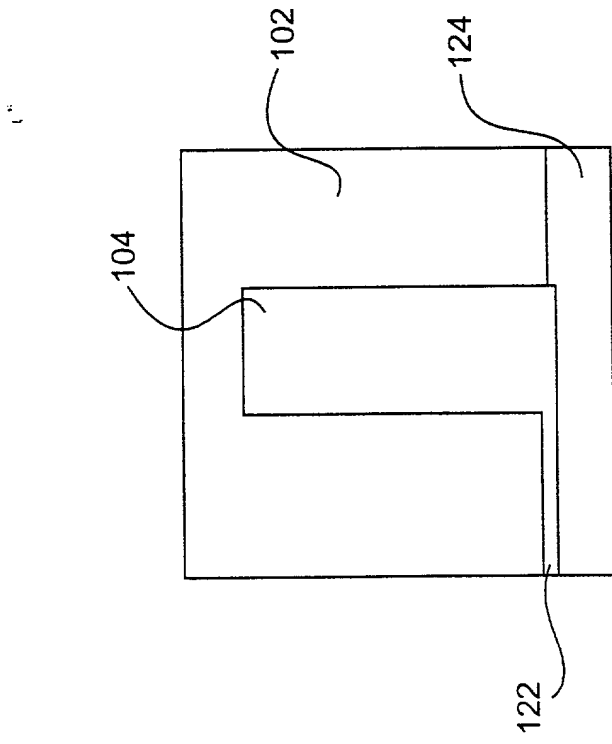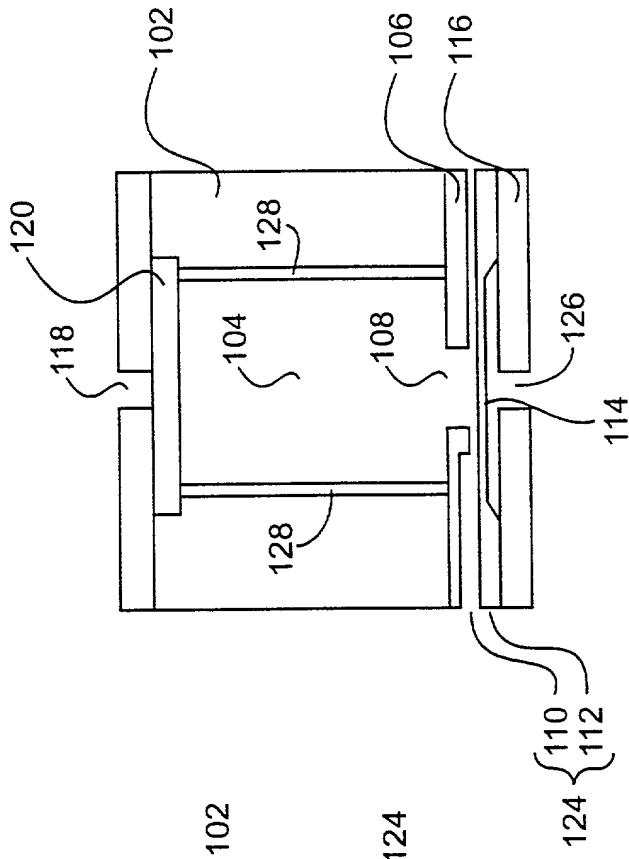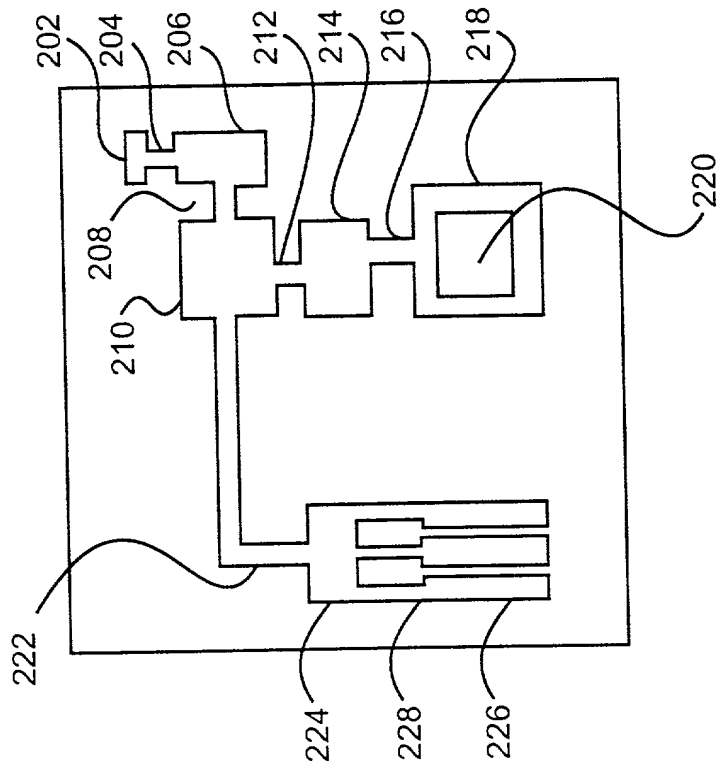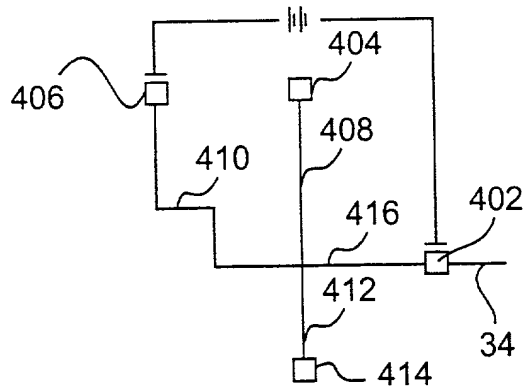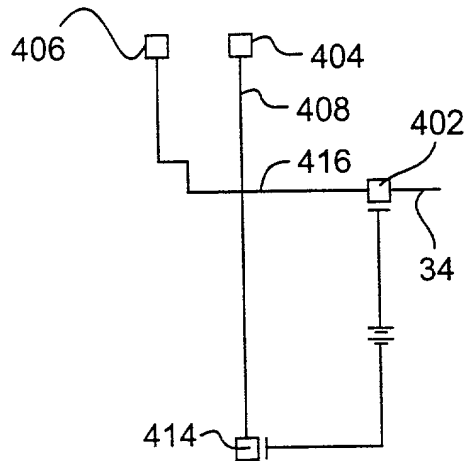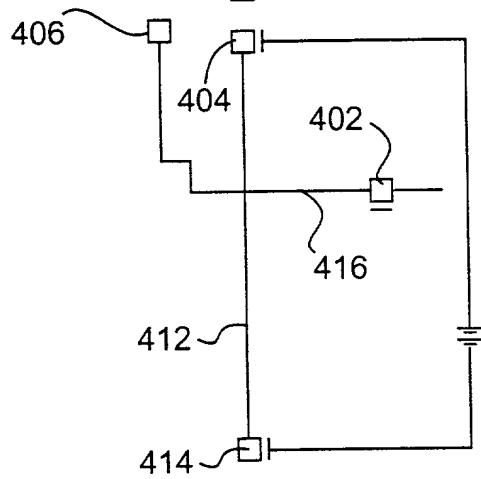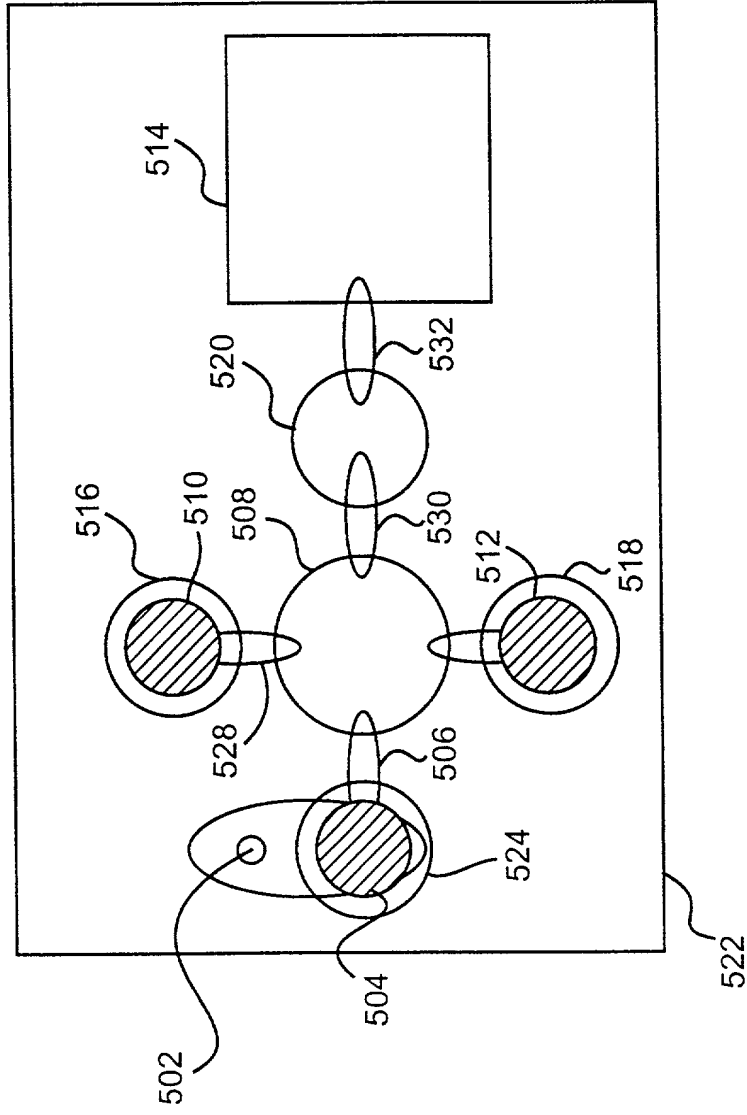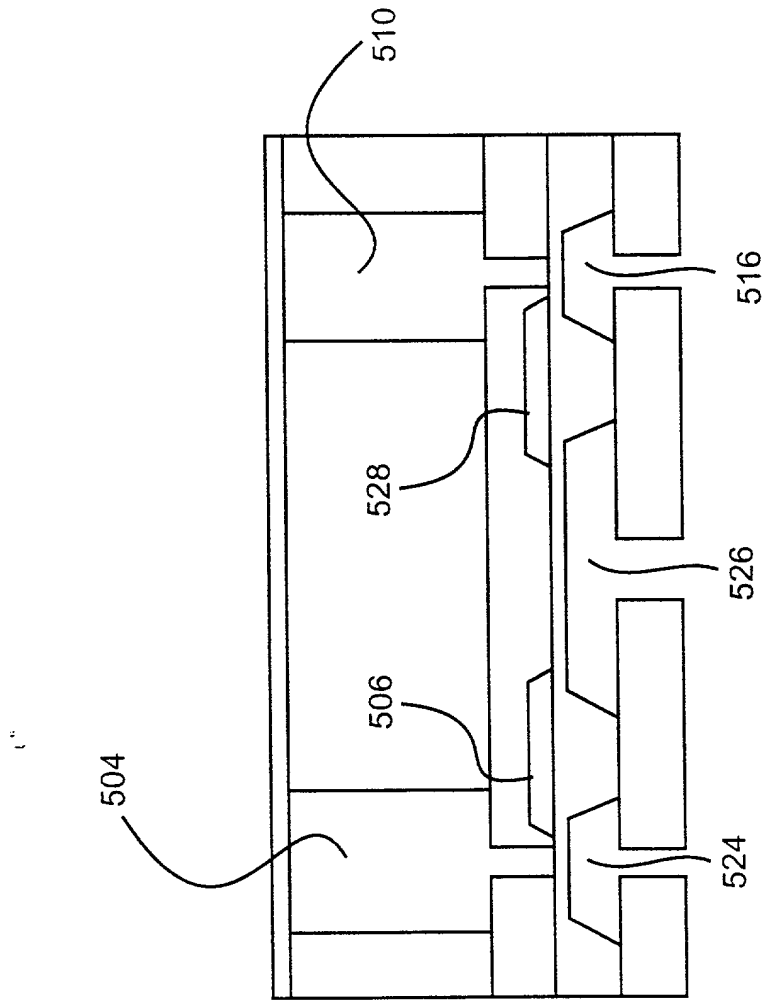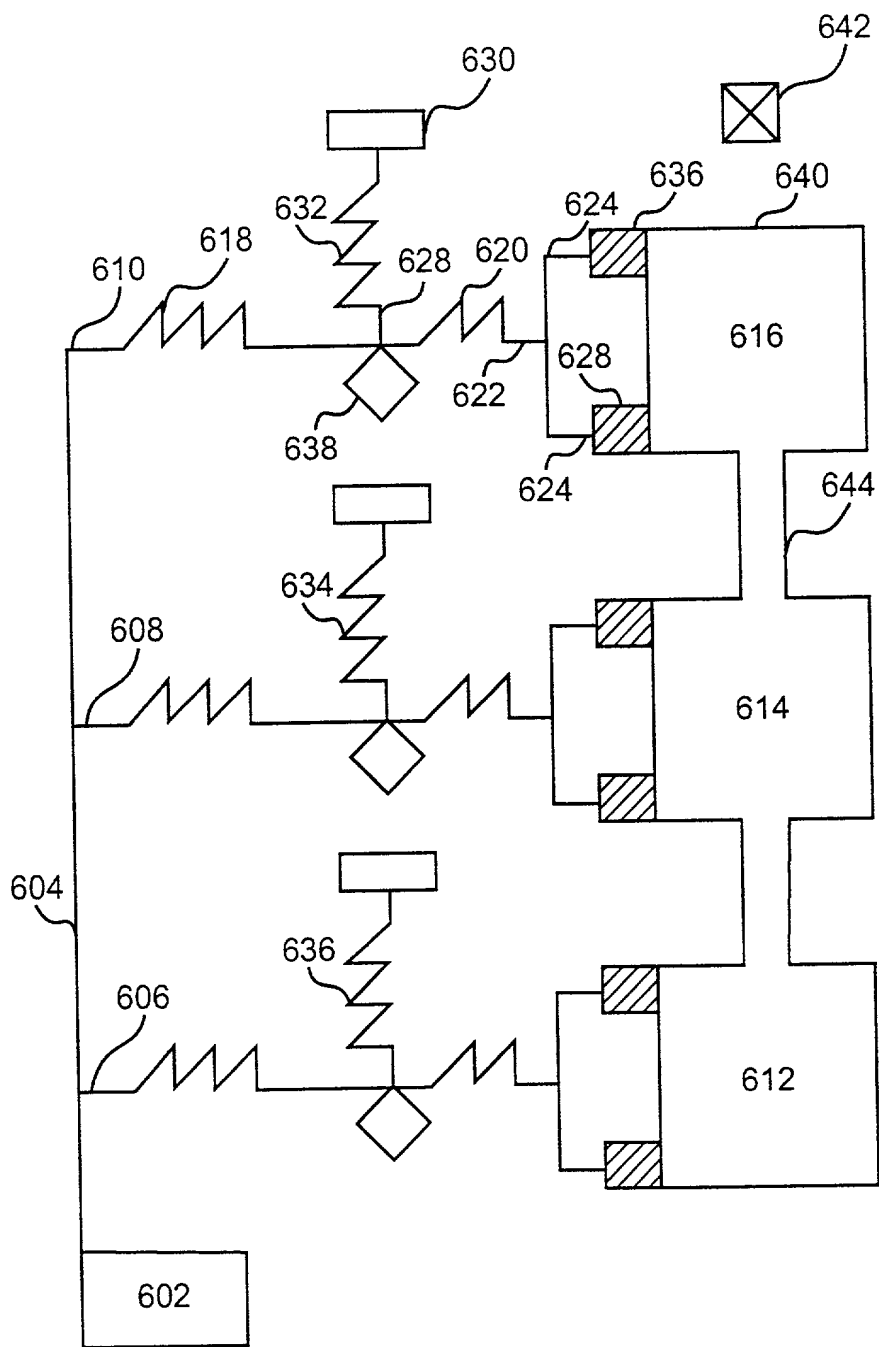FIG. 1

FIG. 2a

FIG. 2b

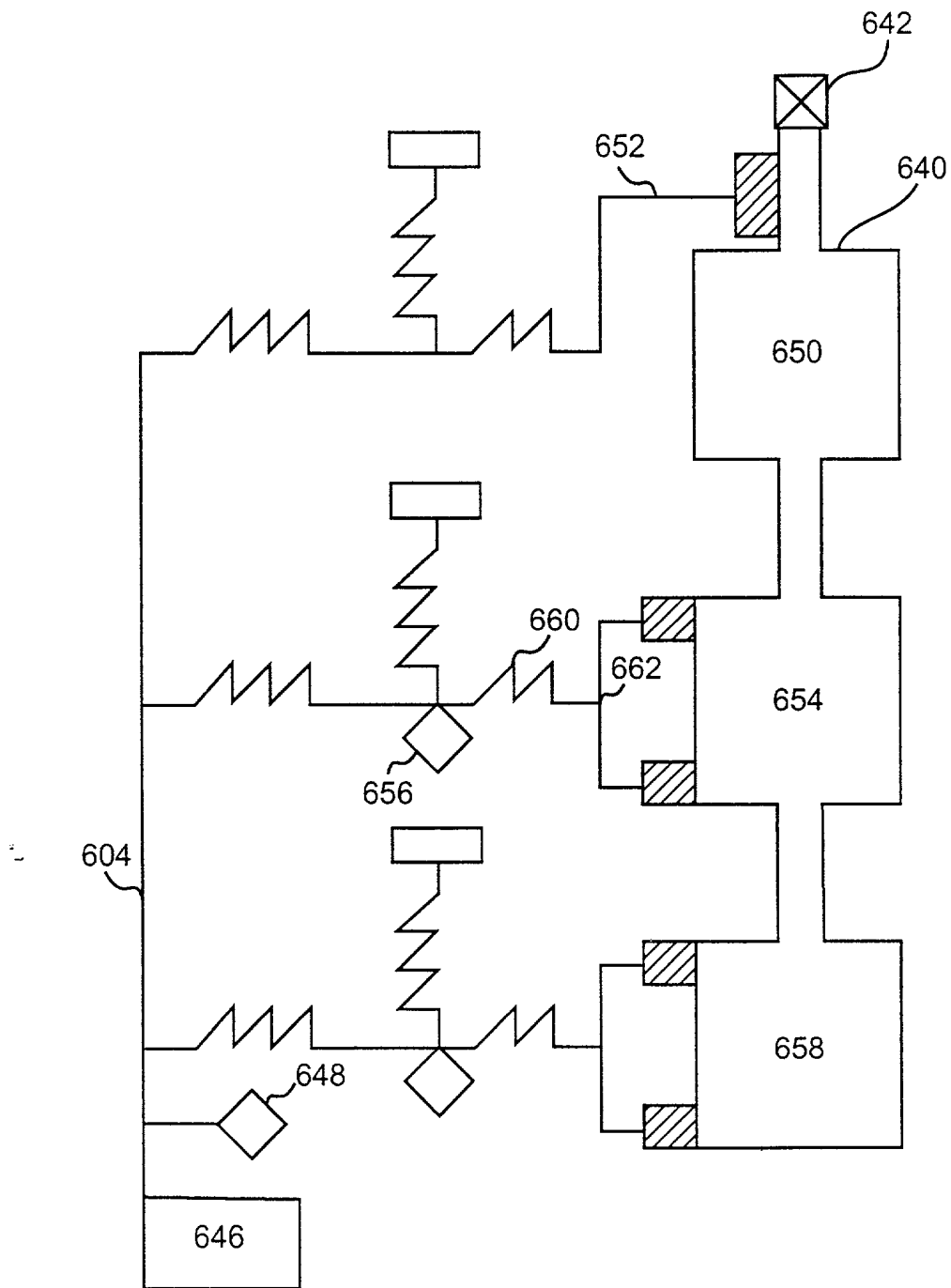FIG. 2a & 2b

FIG. 3

FIG. 4a

FIG. 4b

FIG. 4c

FIG. 4a-4c

FIG. 5a

FIG. 5b

FIG. 6a

FIG. 6b
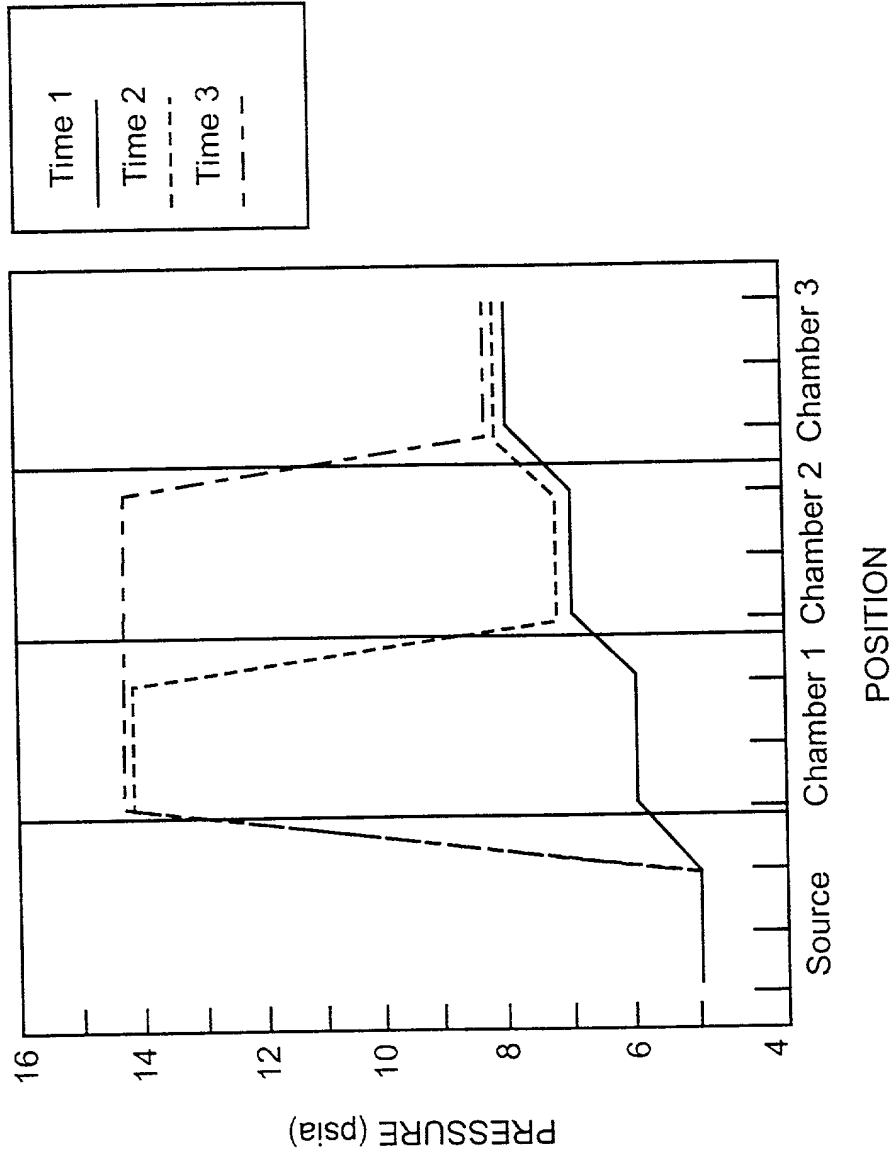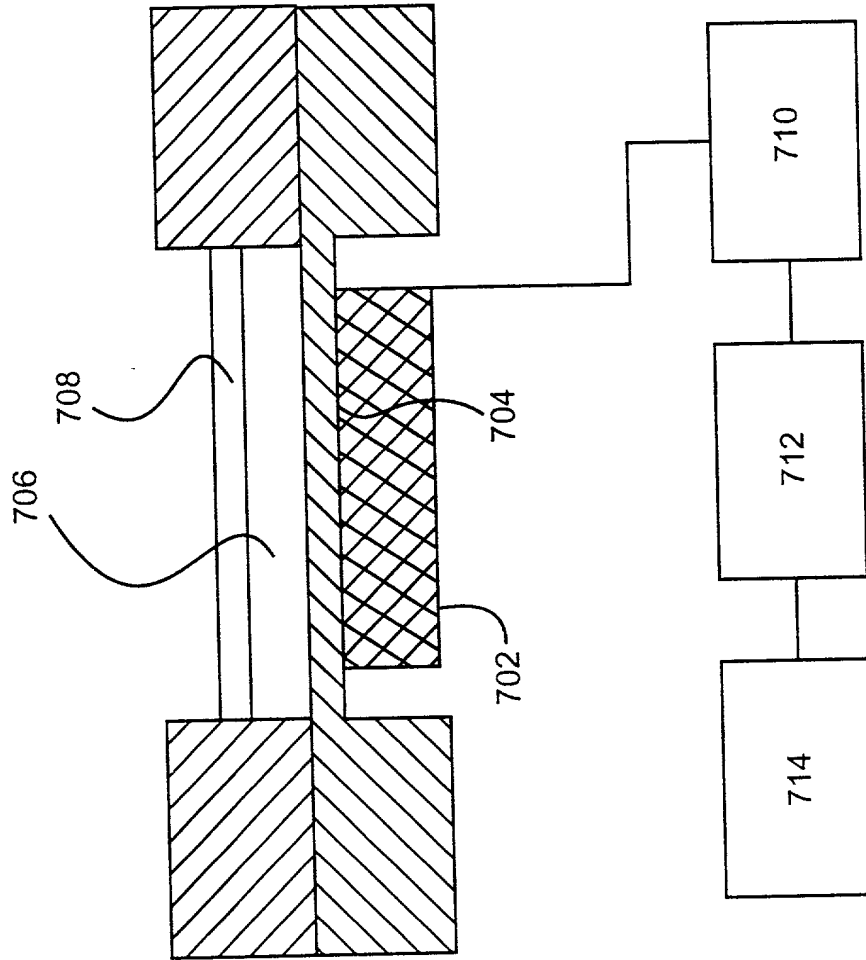
FIG. 6C

FIG. 7a
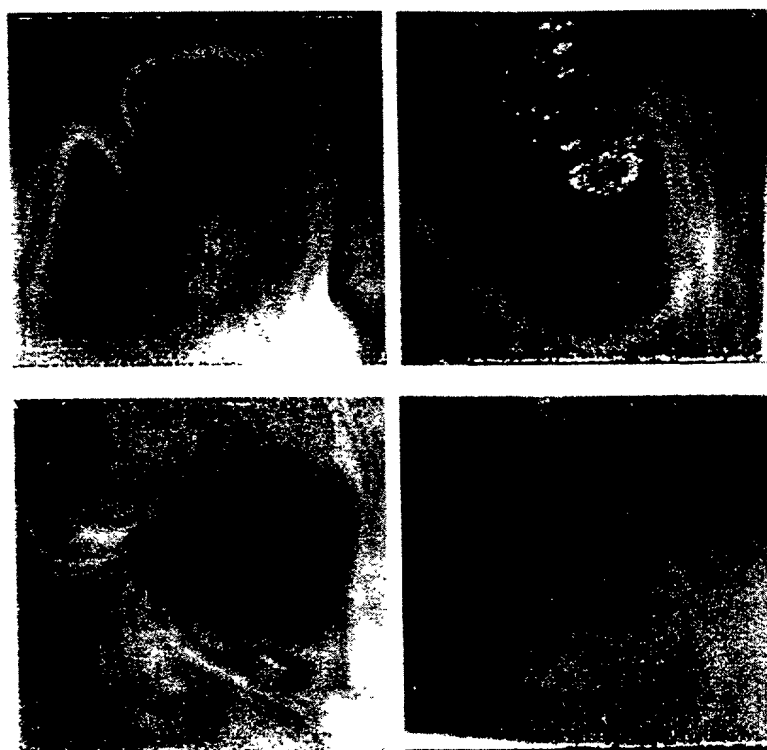
FIG. 7B

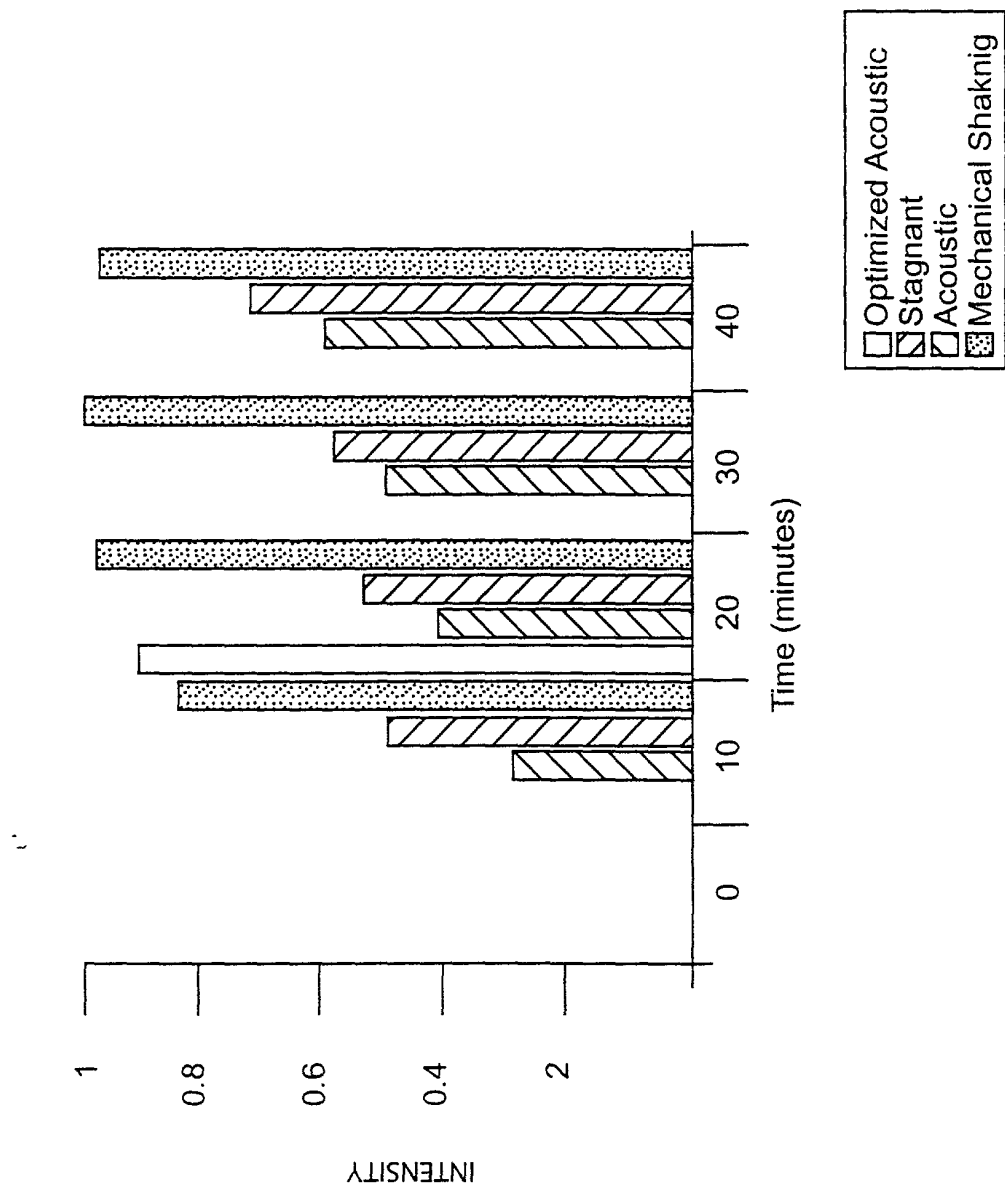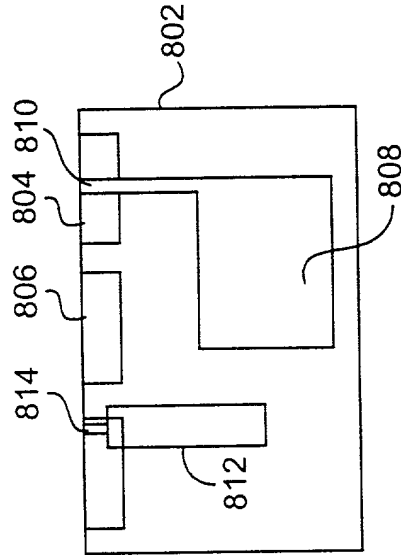FIG. 7c
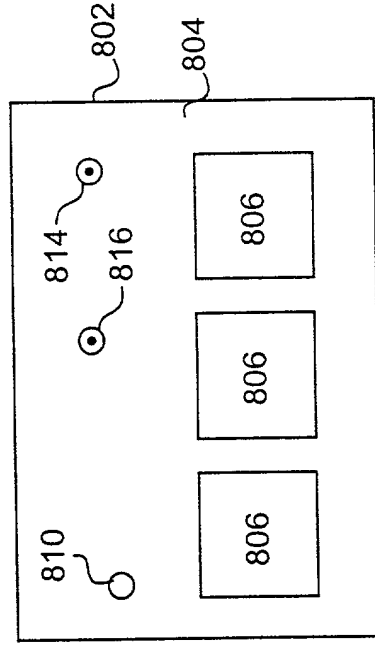
FIG. 8b

FIG. 8a

FIG. 8a & 8b

t =   0   5   10   30   60 120 minutes
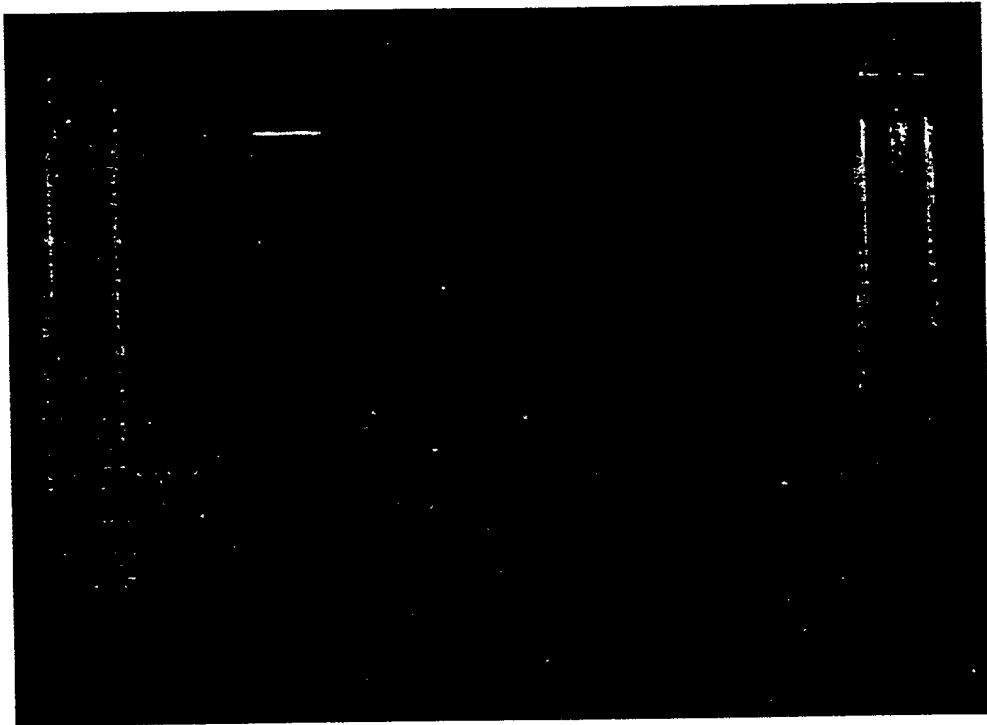
Correct Call Rates:

74%   95.8%   95.9%

95.9%   95.5%   83%

FIG. 9a
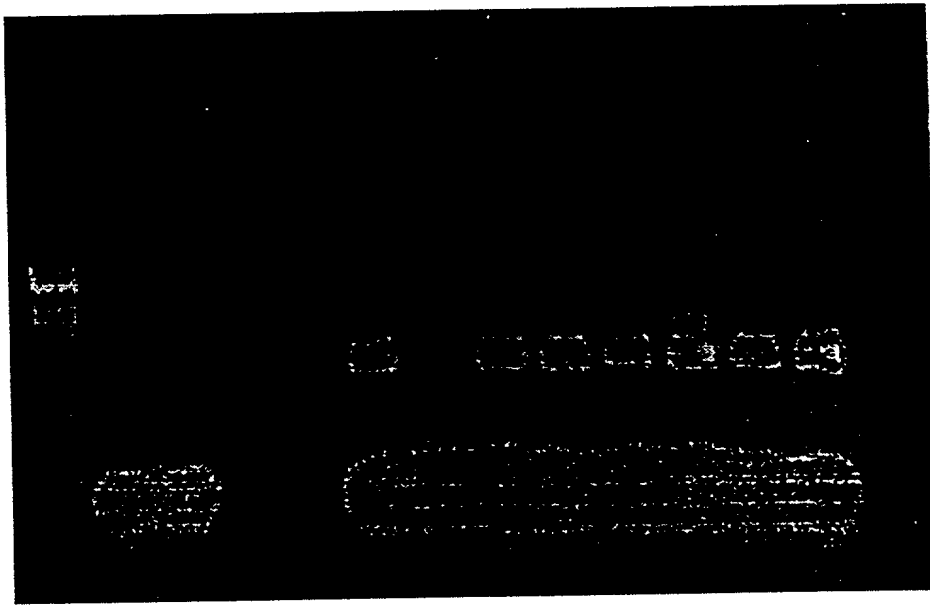
FIG. 9b

FIG. 9c
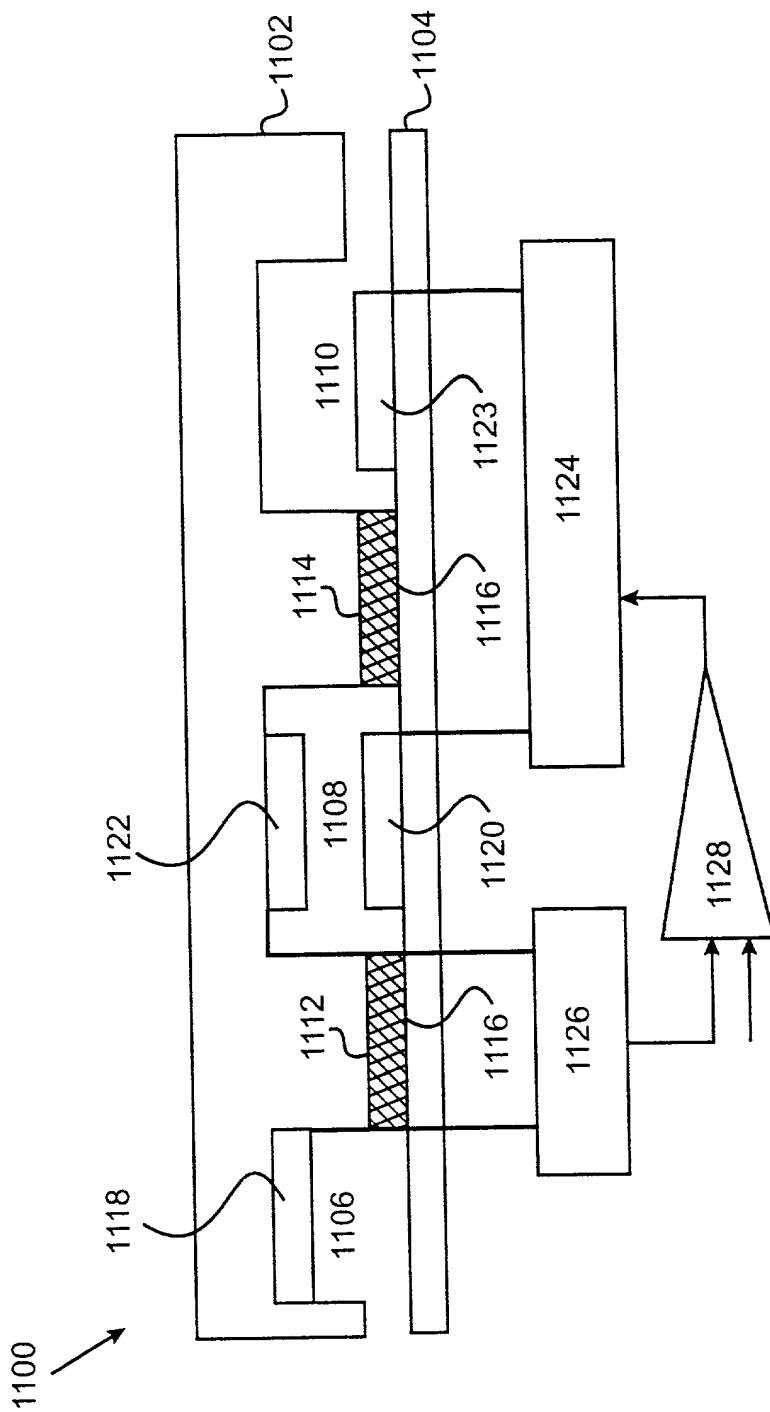
FIG. 10
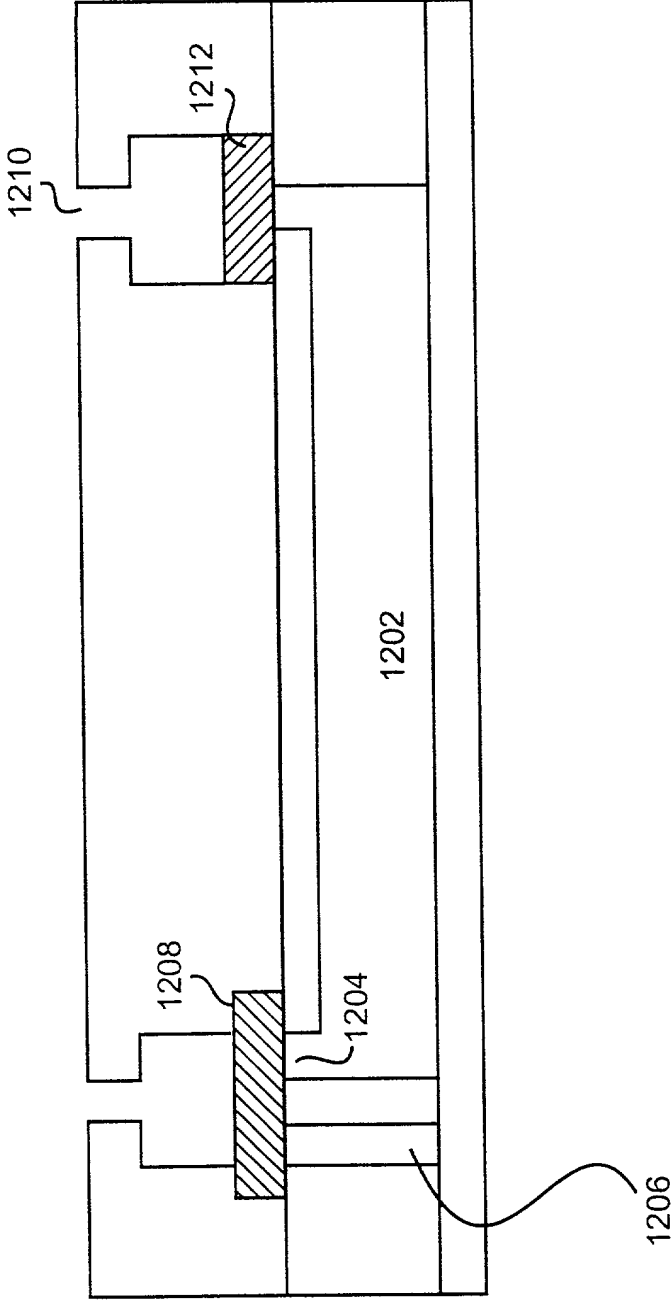
FIG. 11A

FIG. 11b

FIG. 11c

FIG. 11d

Value Array (10)	
Reaction Array	
1 linkage to sample prep array	
5 mix chambers	
2 dose chambers	
1 linker/debubbler	
1 linkage to storage array	

Value Array (10)	
Reaction Array	
1 linkage to sample prep array	
5 linkage to post processing	
5 Storage Chambers	
2 dose chambers	
1 reagent inlet	

Value Array (10)	
Sample Prep Array	
1 linkage to reaction array	
1 sample inlet	
5 reagent inlets	
2 rxn chambers	
1 storage chamber	

Value Array (10)	
Post-Processing	
1 linkage to reaction array	
1 sample inlet	
5 reagent inlets	
2 rxn chambers	
1 storage chamber	

DNA Array and/or Electrophoresis Channels

FIG. 12

FIG. 13

FIG. 14a

FIG. 14b

FIG. 15

FIG. 16

FIG. 17a

1803

1703

1702

S1 S2 R1 R2 IN1 IN2

1704 1706 1801

1706 1801

FIG. 17b

1802

1802

1722

1724

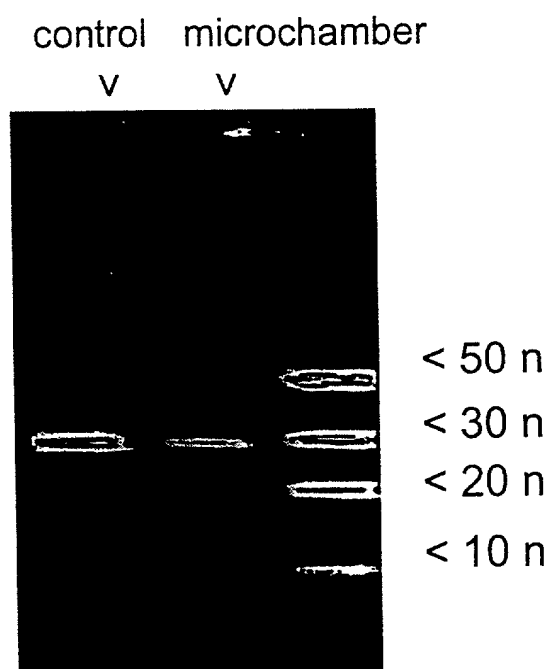1701

1802 1802 1726 1728

FIG. 17

1906 1910 1902

1901

1908 1912

1920 1922

1900

FIG. 18
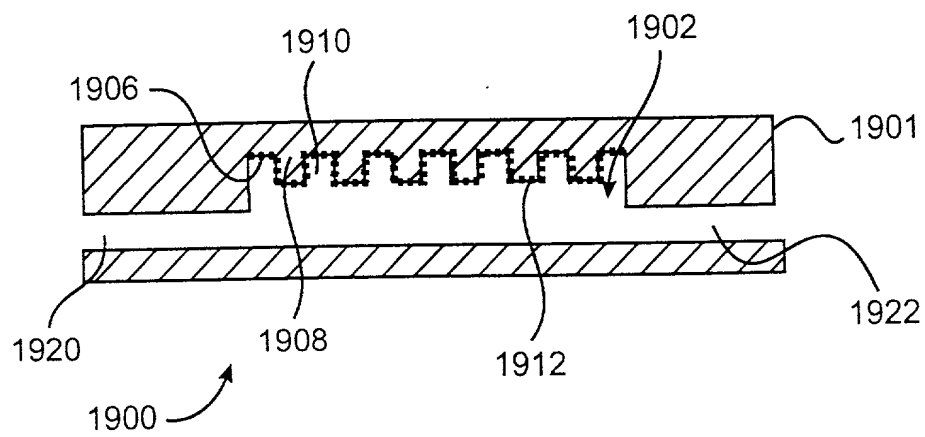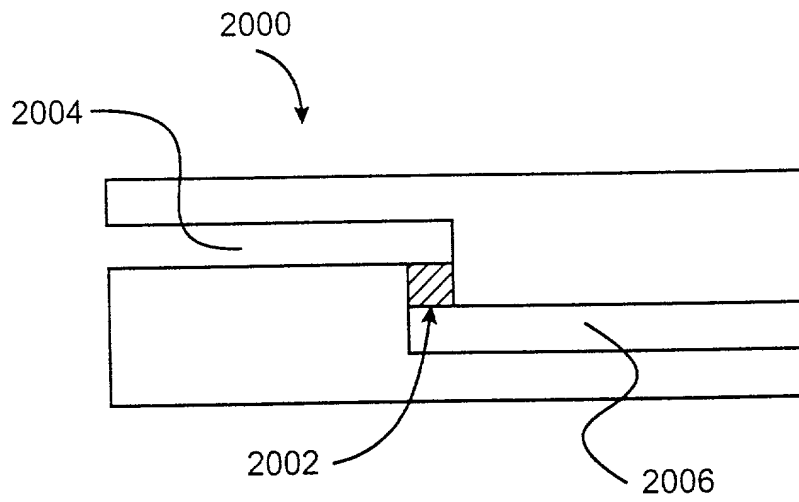
FIG. 19
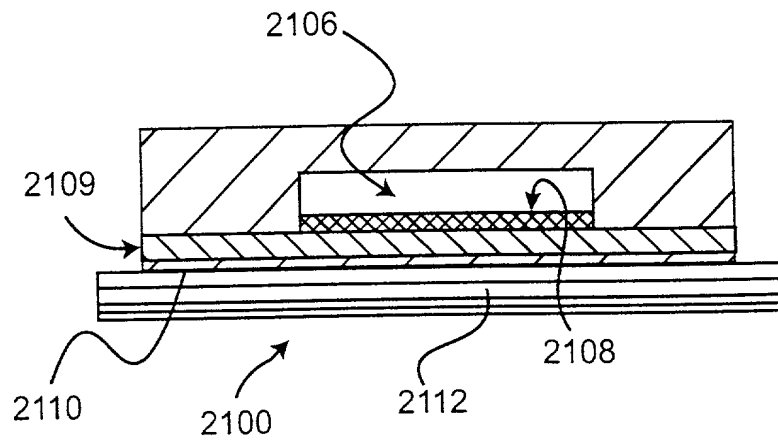
FIG. 20
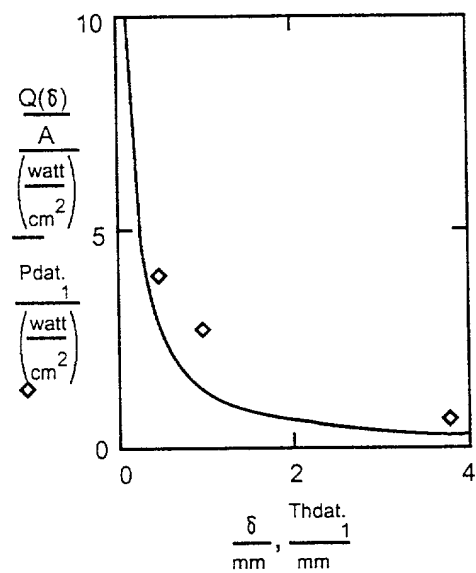
FIG. 21a

FIG. 21b

FIG. 22

FIG. 23
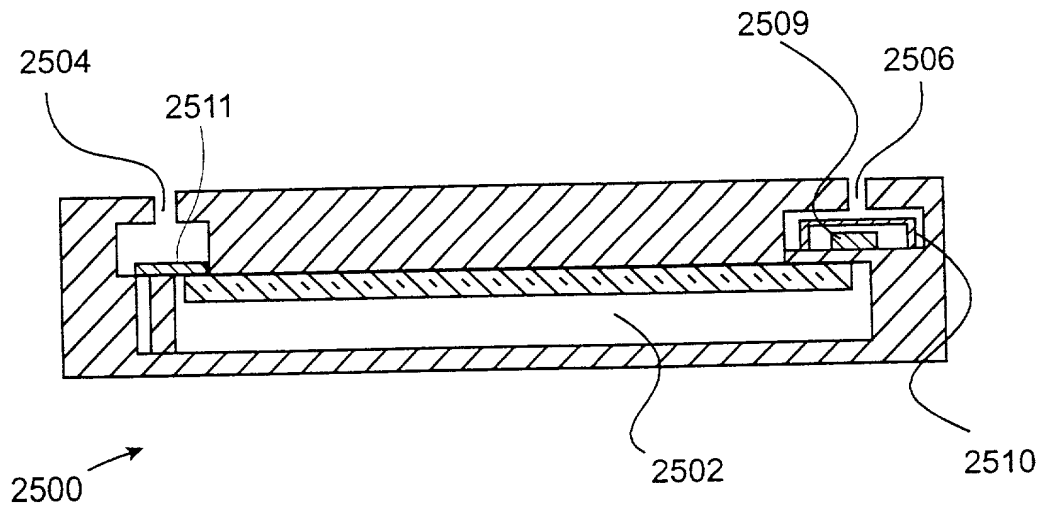
FIG. 24
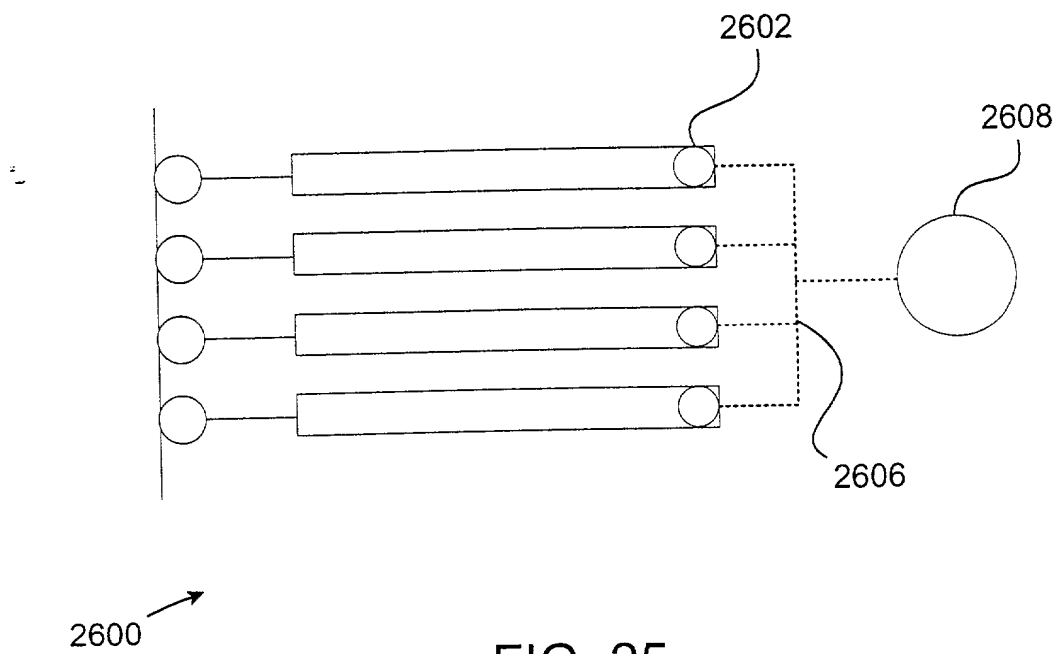
FIG. 25
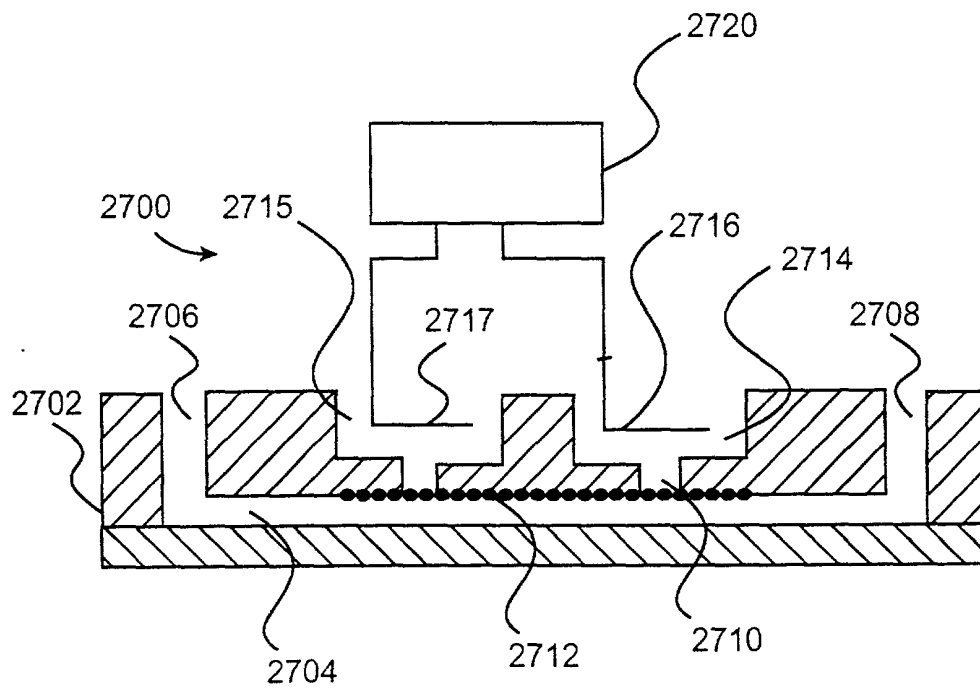
FIG. 26
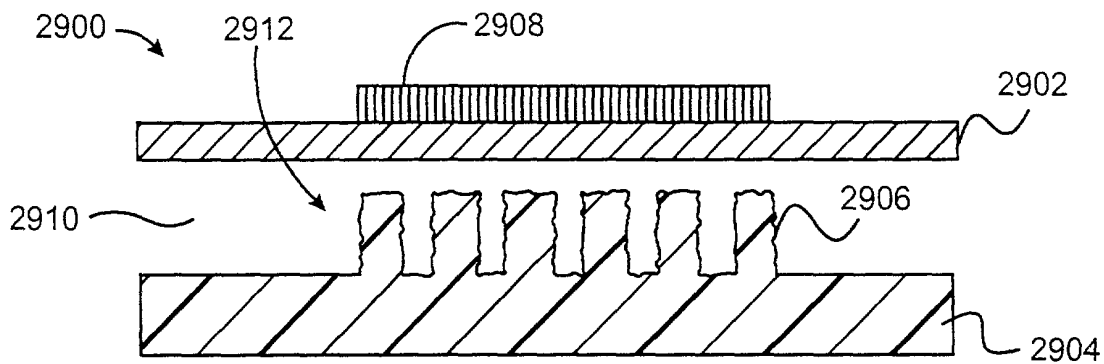
FIG. 27
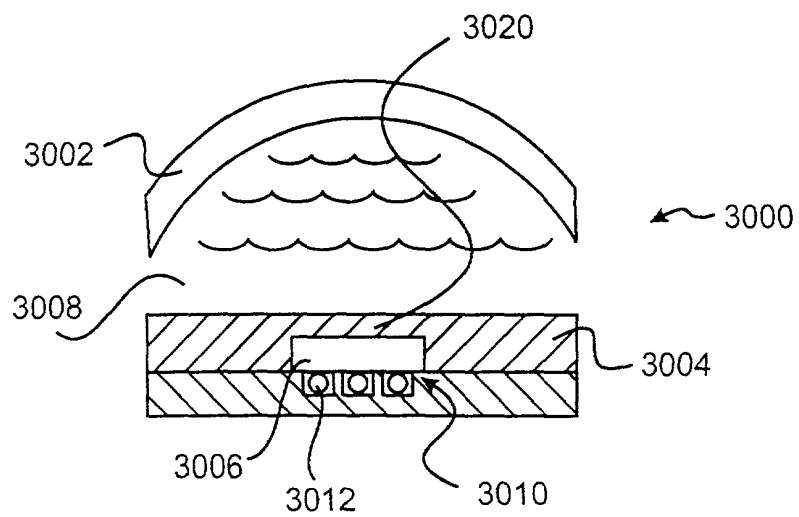
FIG. 28
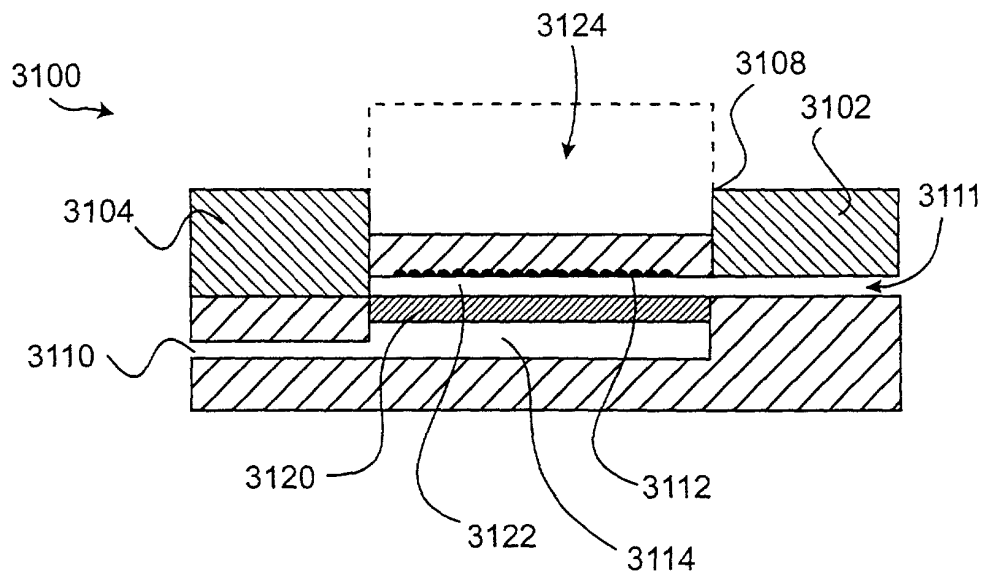
FIG. 29
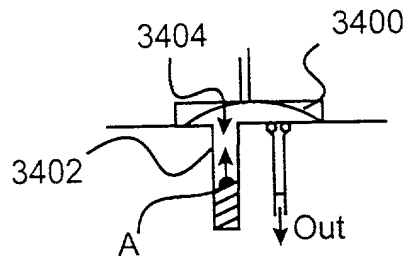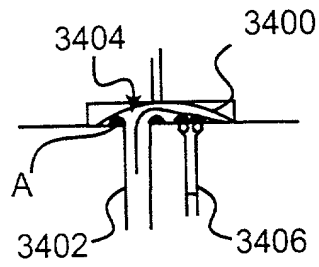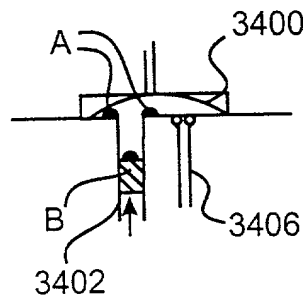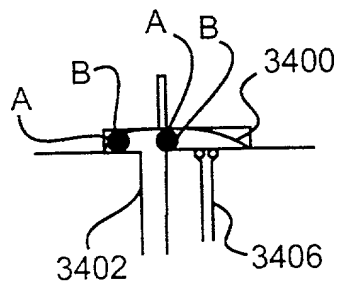
3404   3400

3402

A   Out

FIG. 30a

3404   3400

A

3402   3406

FIG. 30b

A   3400

B

3402   3406

FIG. 30c

A   B

B   3400

A

3402   3406

FIG. 30d

3400

A,B

3402   3406

FIG. 30e

FIG. 31a

FIG. 31b

3800

FIG. 32a

3802

3800

3804

3806

FIG. 32b

3803

3800

3804

3806

FIG. 32c

3802

3800

3810

3804

3806

FIG. 33a

3802

3800

3810

3806

FIG. 33b

3802

3800

3810

3806

FIG. 33c

3802

3800

3810

3806

FIG. 33d

3810

3806

3804
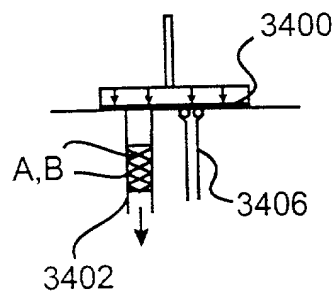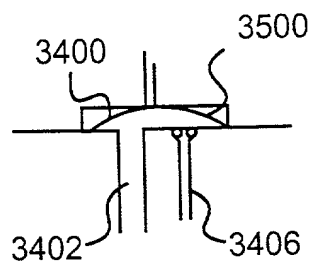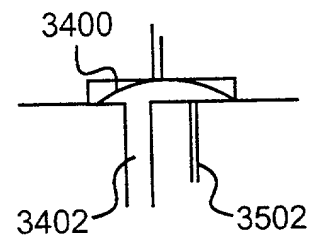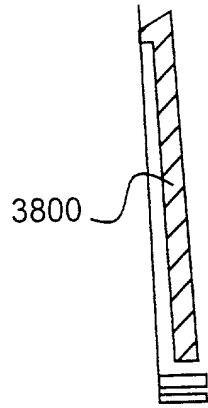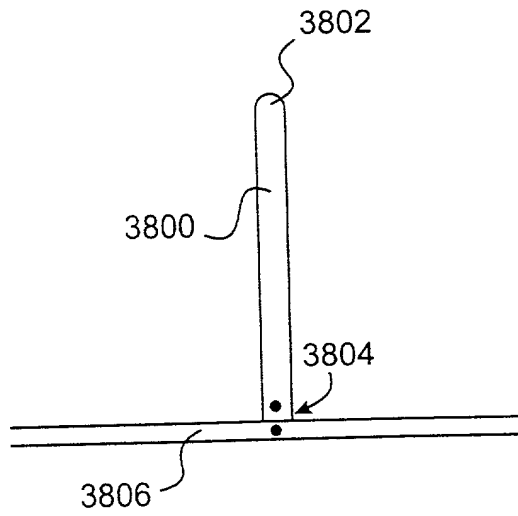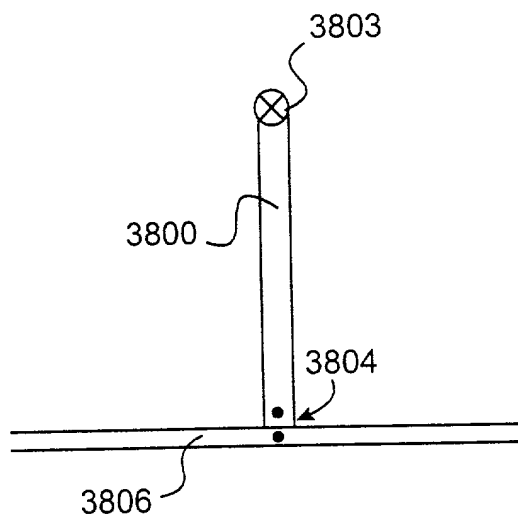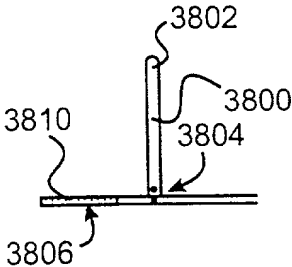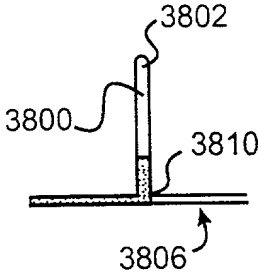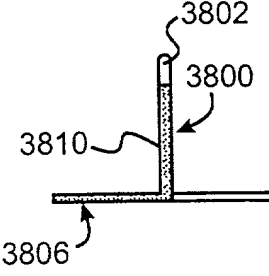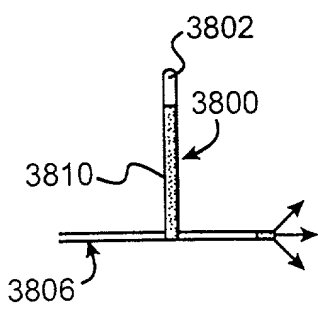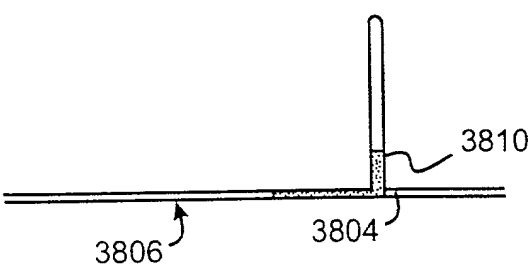
FIG. 33e
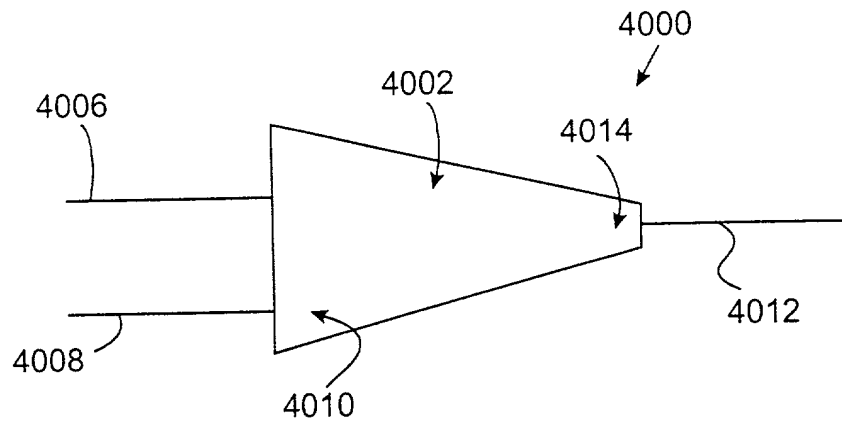
FIG. 34
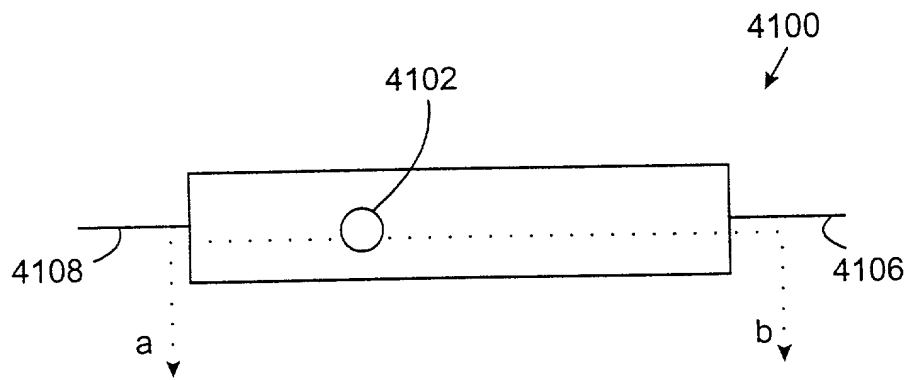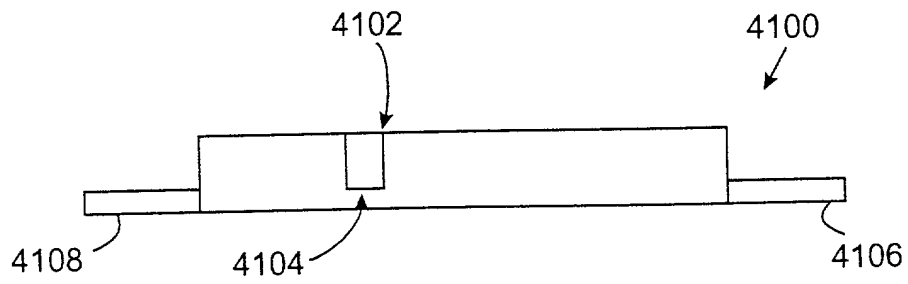
FIG. 35a

FIG. 35b
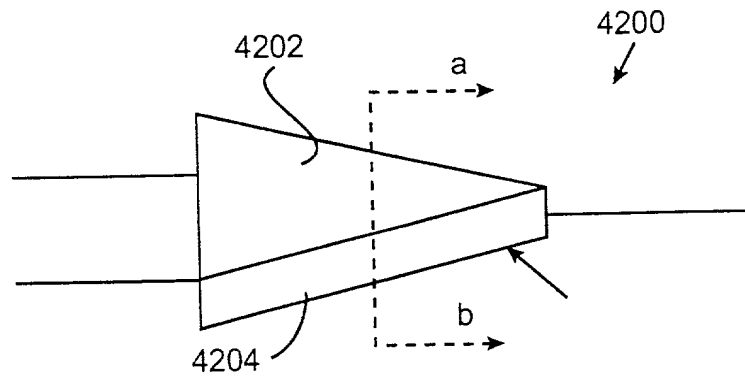
FIG. 36a
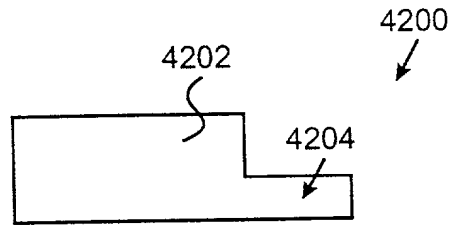
FIG. 36b

FIG. 37a

FIG. 37b

4500

4502

4506

4504
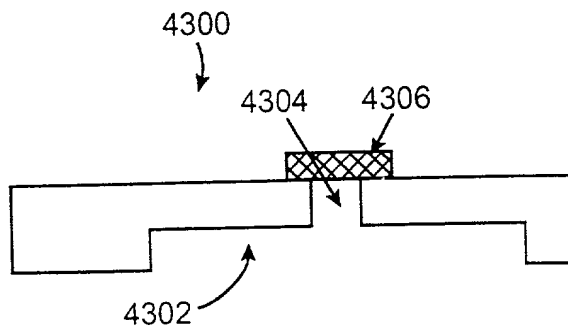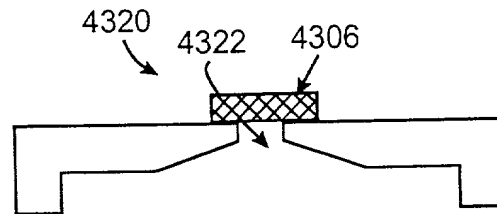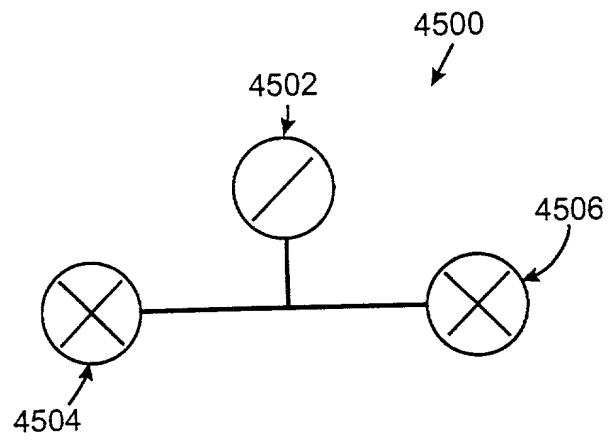
FIG. 38
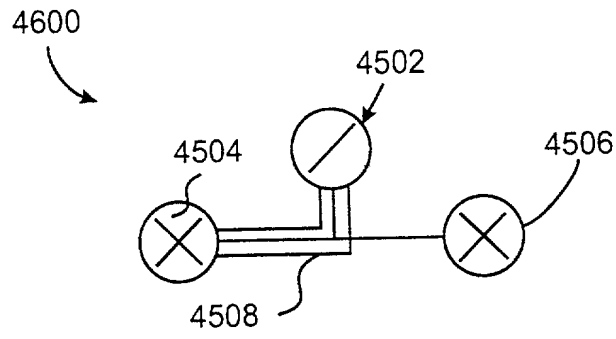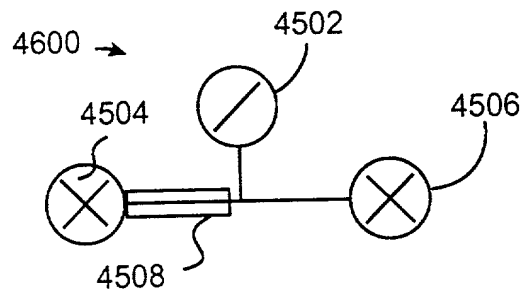
FIG. 39a

FIG. 39b
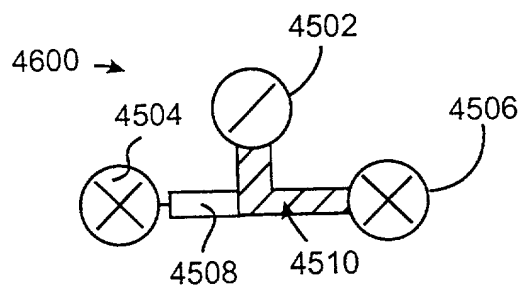
FIG. 39c
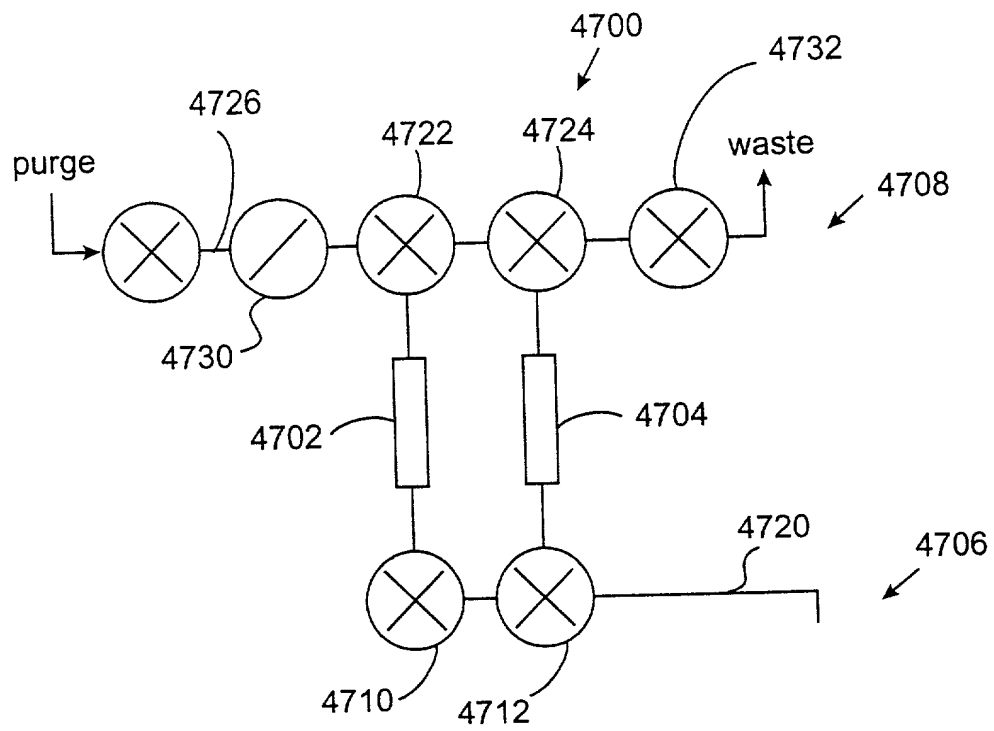
FIG. 40
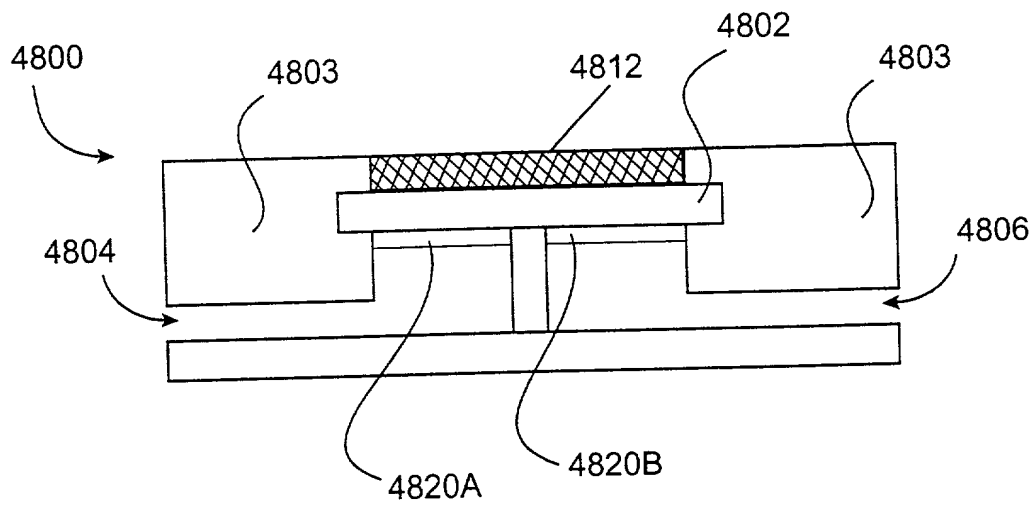
FIG. 41
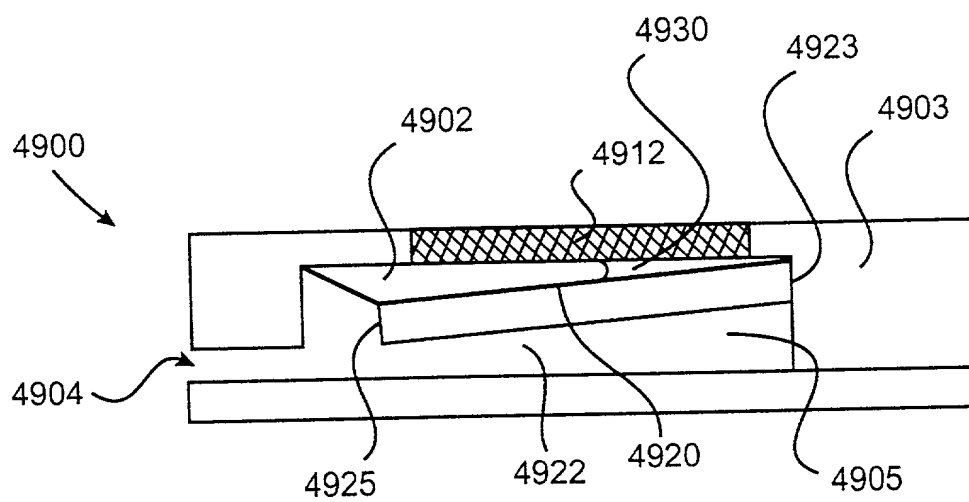
FIG. 42
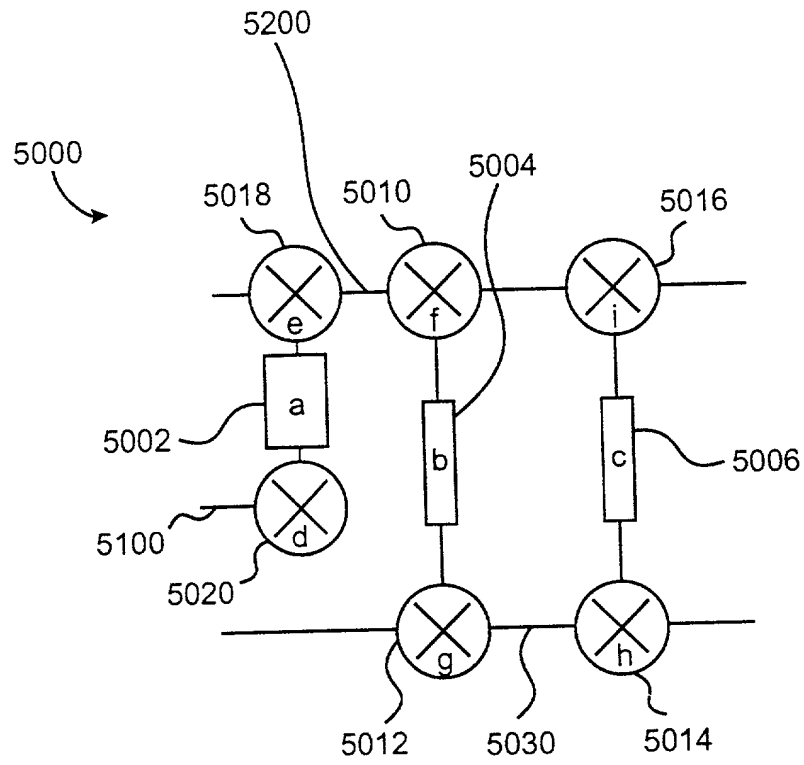
FIG. 43
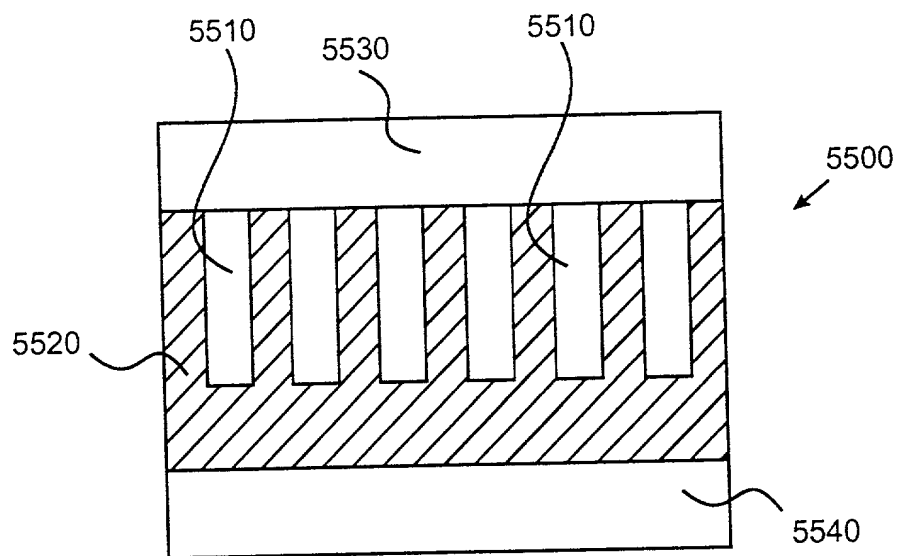
FIG. 44a
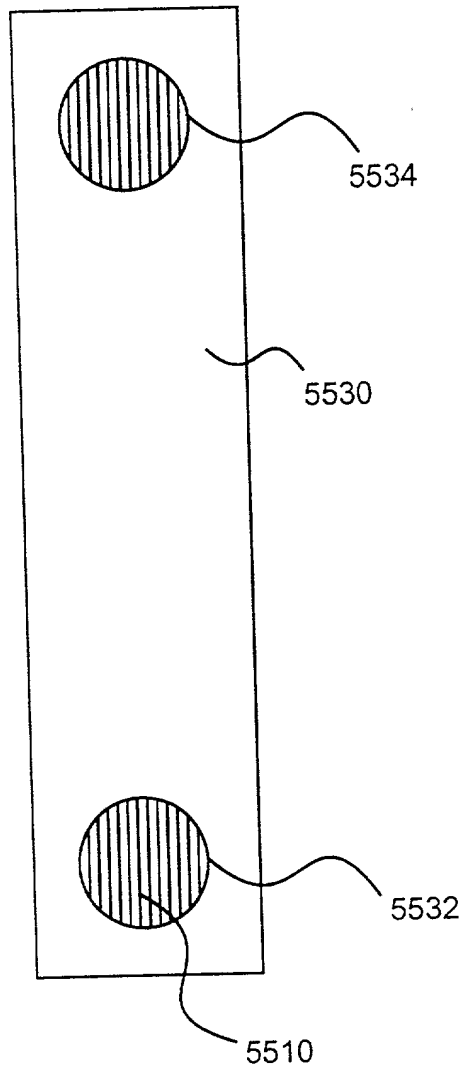
FIG. 44b

Transient heating in water
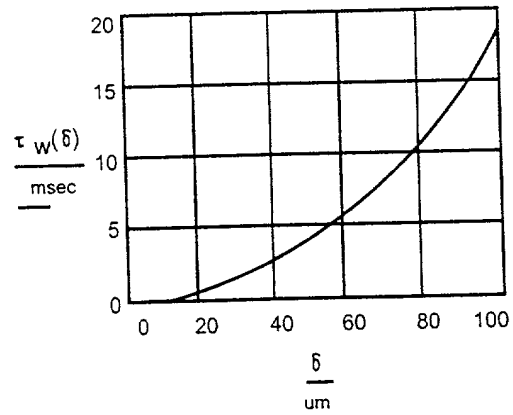
FIG. 44c

Half-gap required in flow-through heating structure
(water, L=1 mm, w=10, 100, 500 um)

FIG. 44d

5603   5604

5602

FIG. 45a

5602

5606

5608

FIG. 45b

5610

5606

5608

FIG. 45c

FIG. 46a

FIG. 46b

5810 5816 5804 5811 5802

5807

5805 5804 5805

5806

FIG. 47a

5802

5807

5810 5804
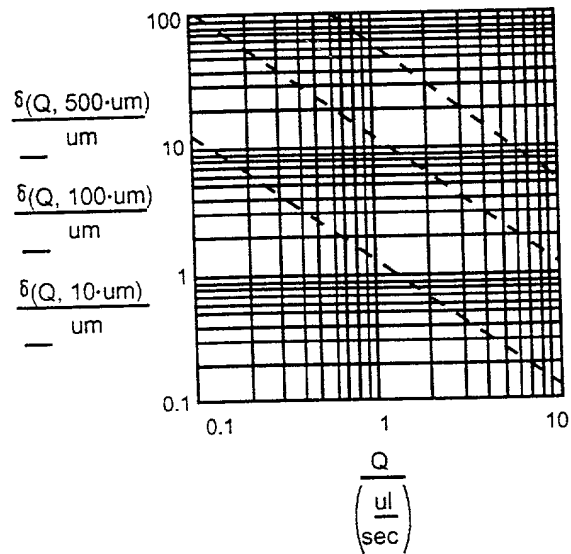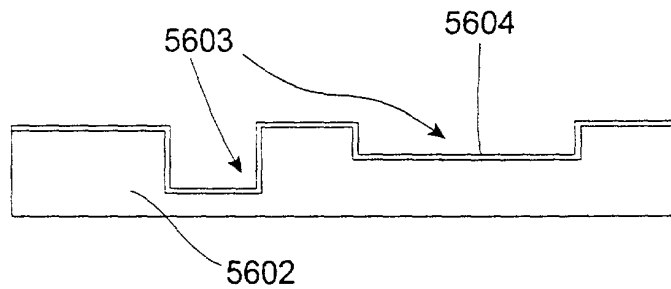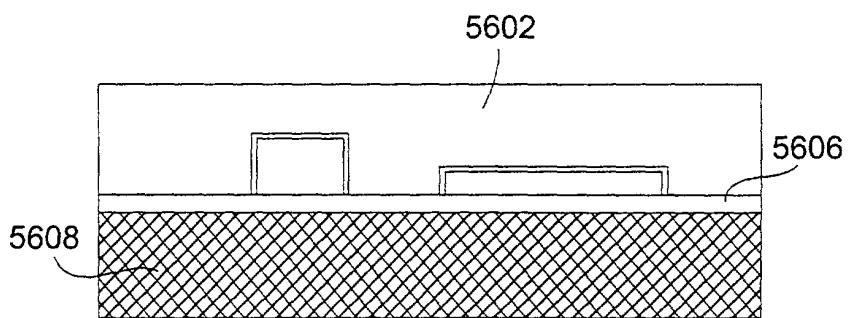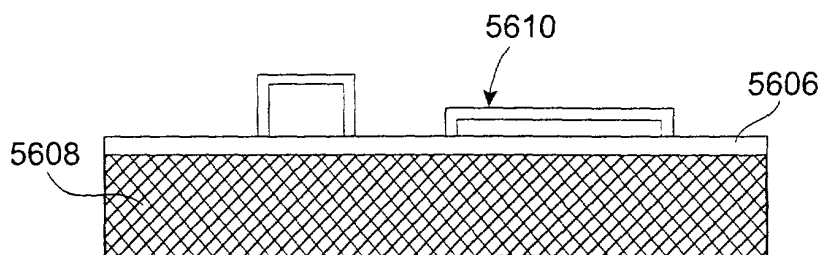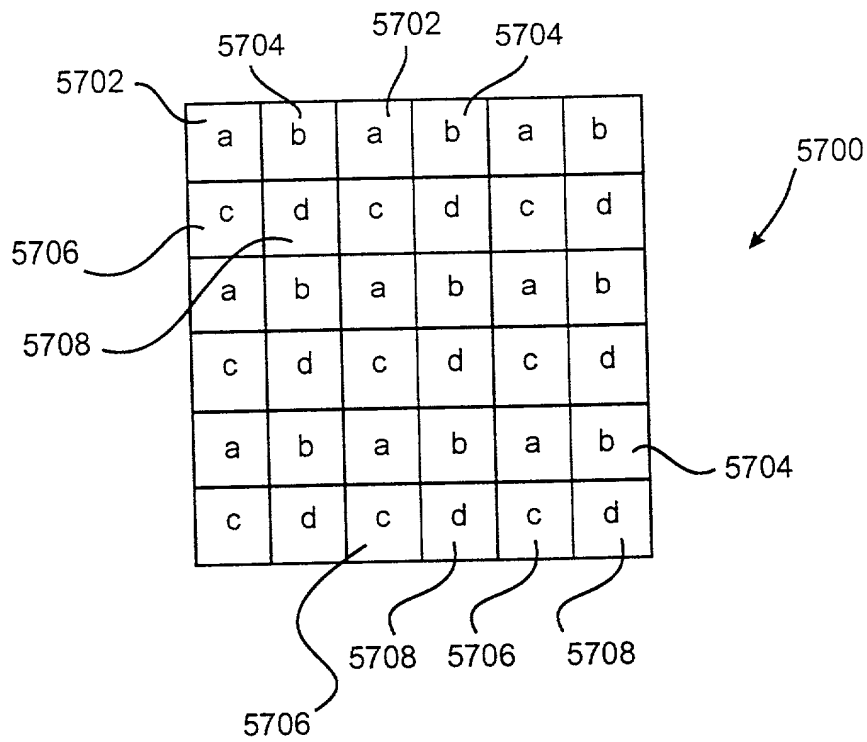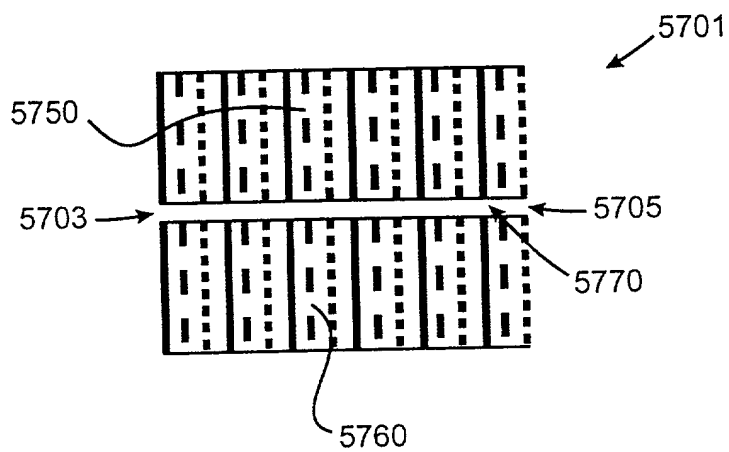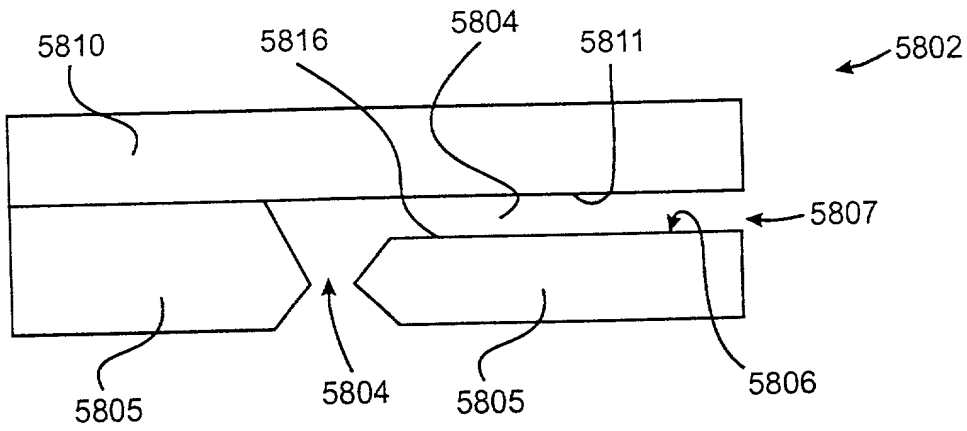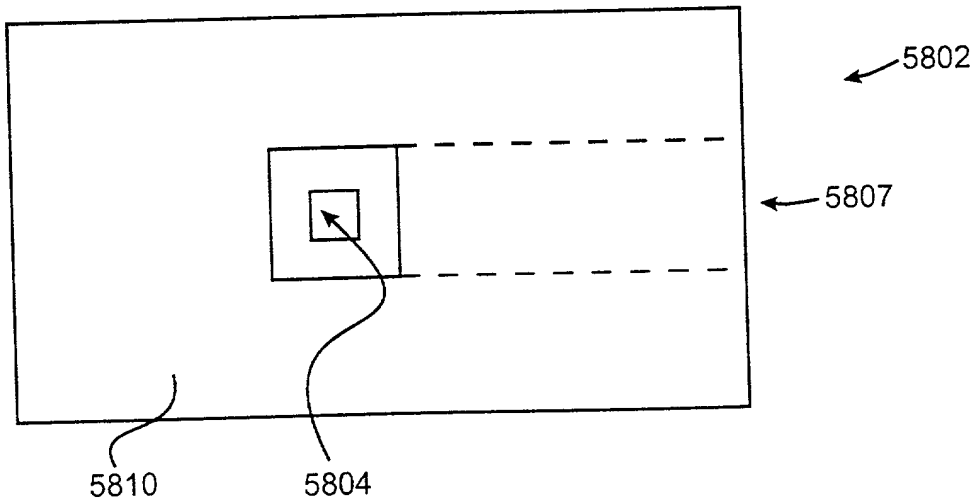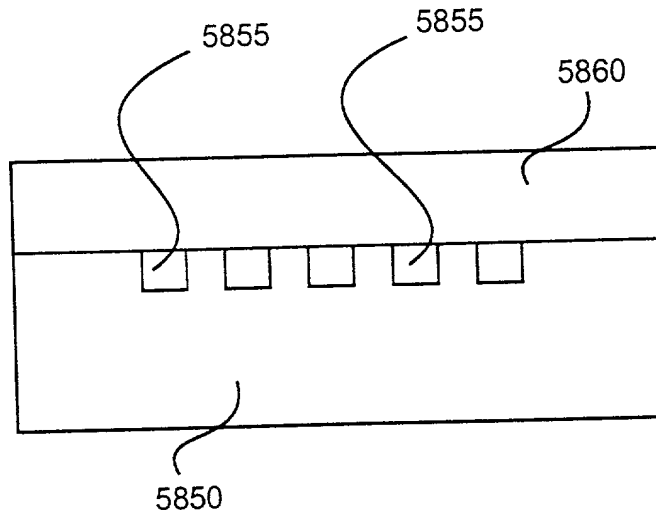
FIG. 47b

FIG. 47c

FIG. 48

FIG. 49

FIG. 50

6215

6200

6215

6215
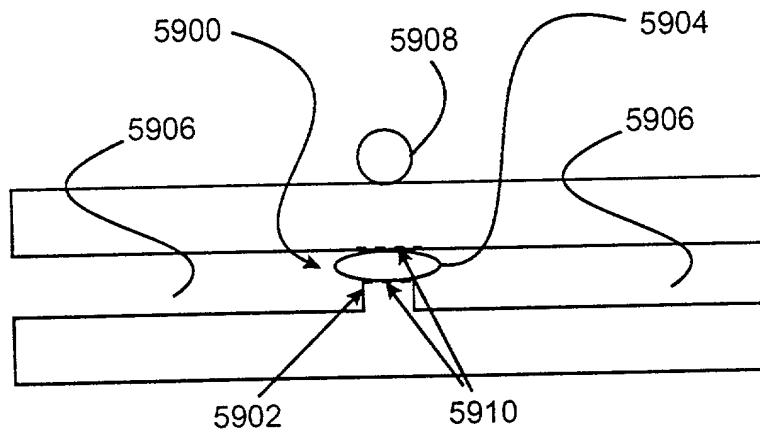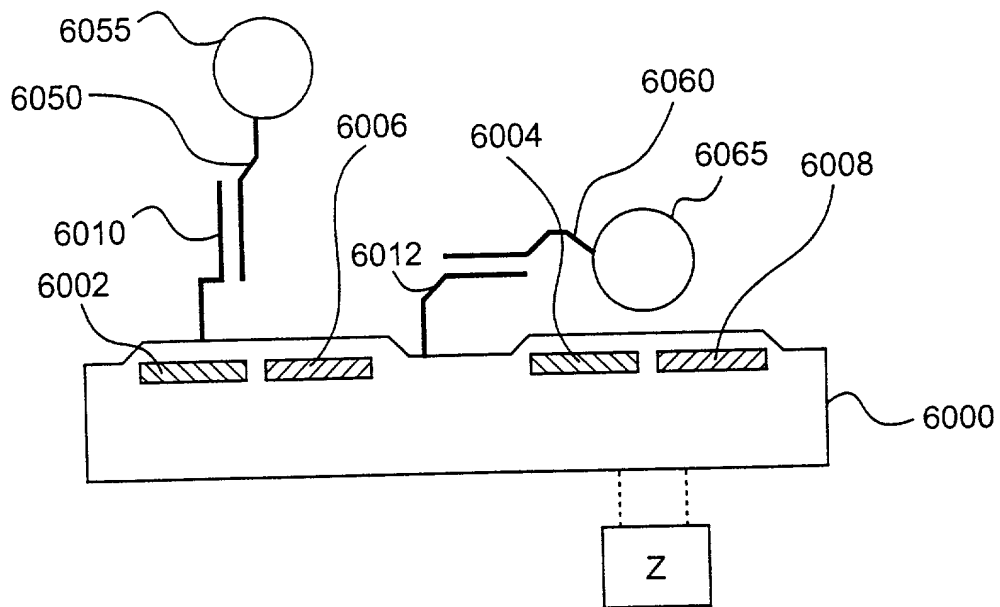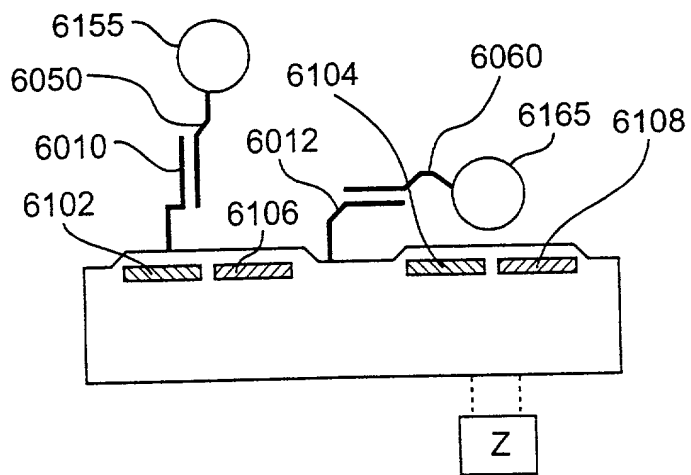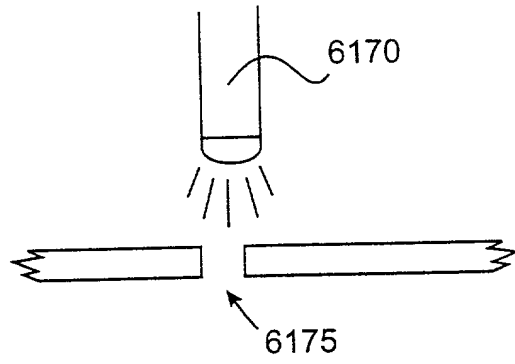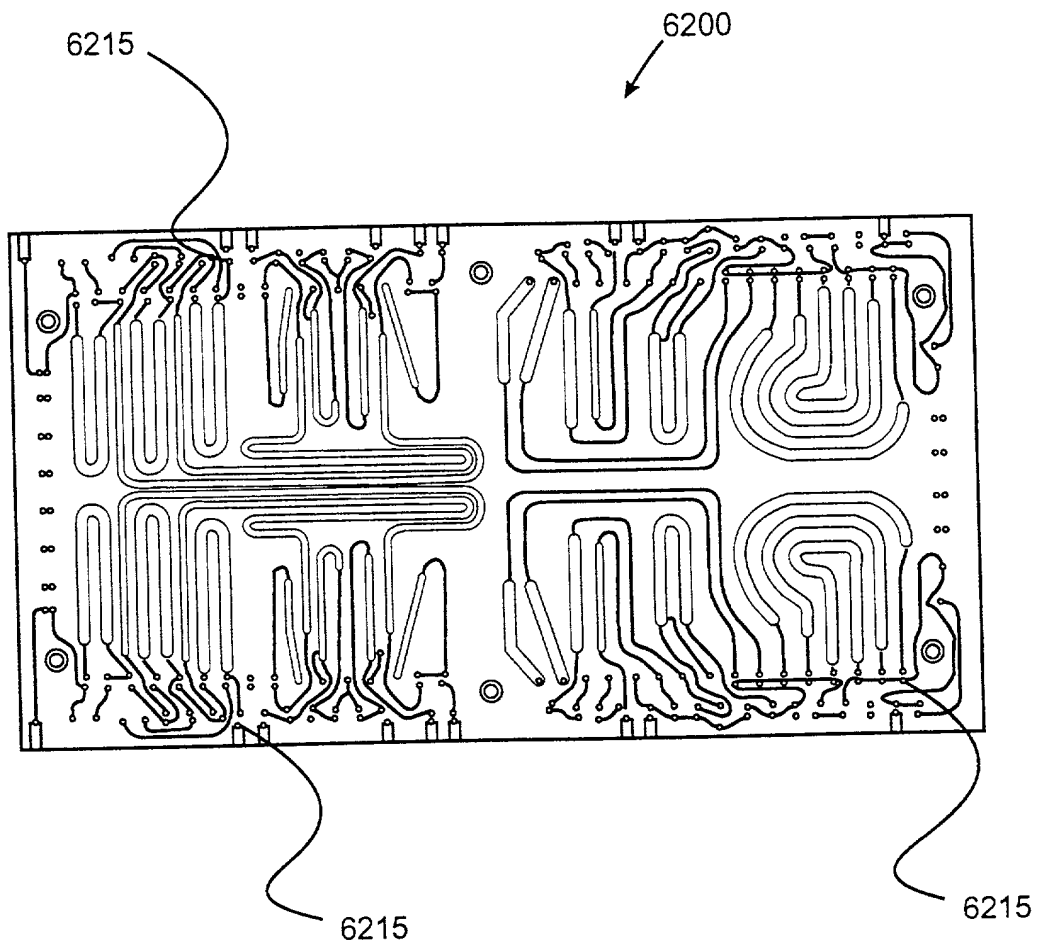
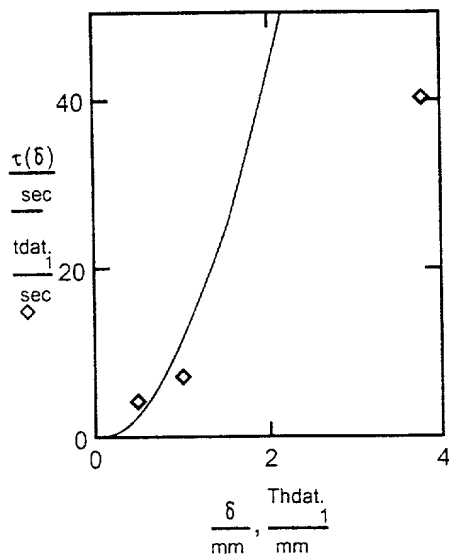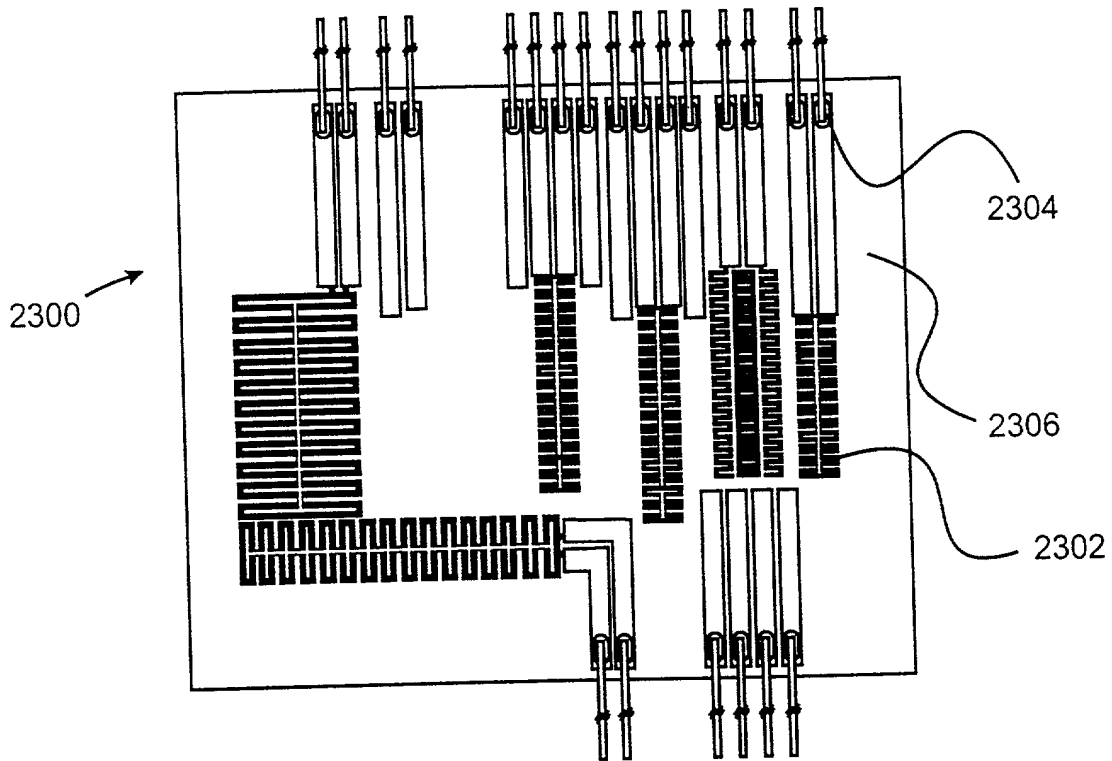FIG. 51
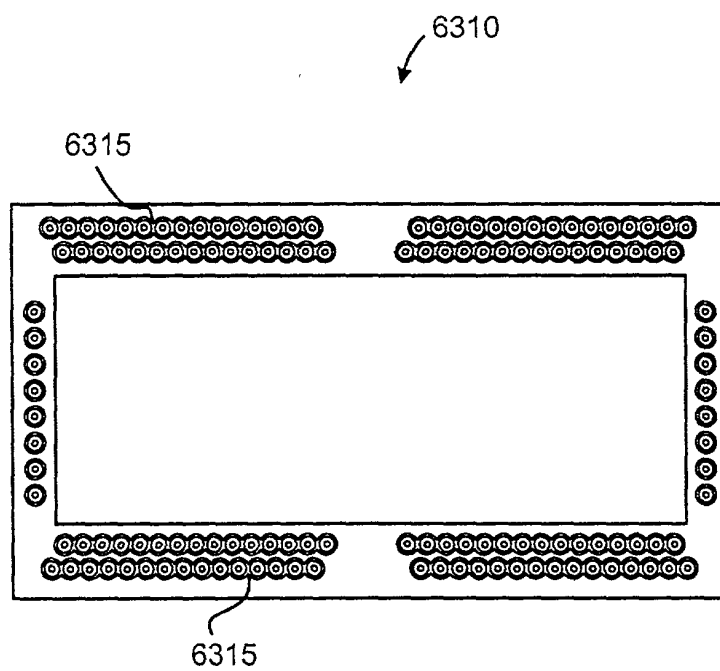
FIG. 52
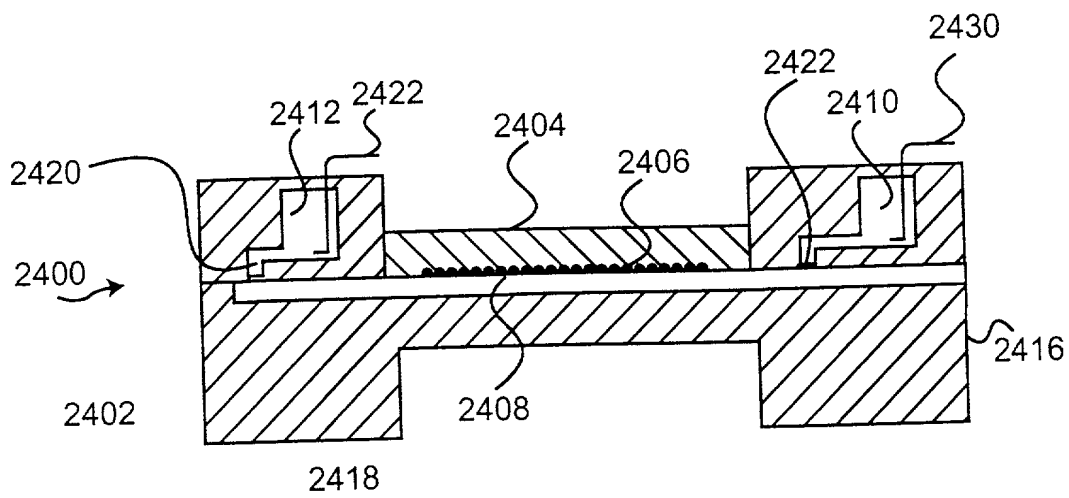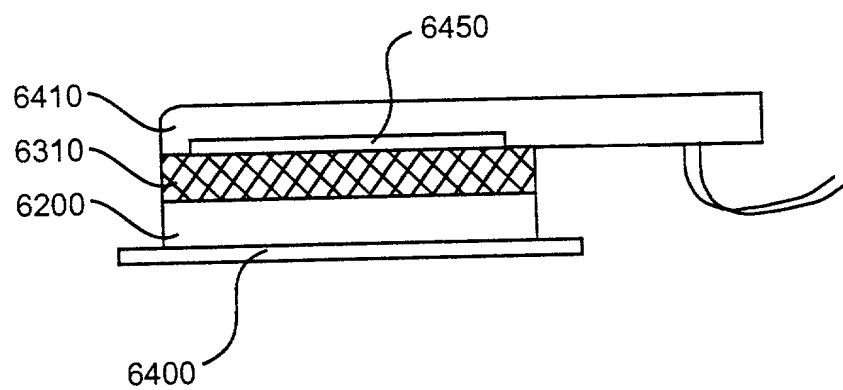
FIG. 53
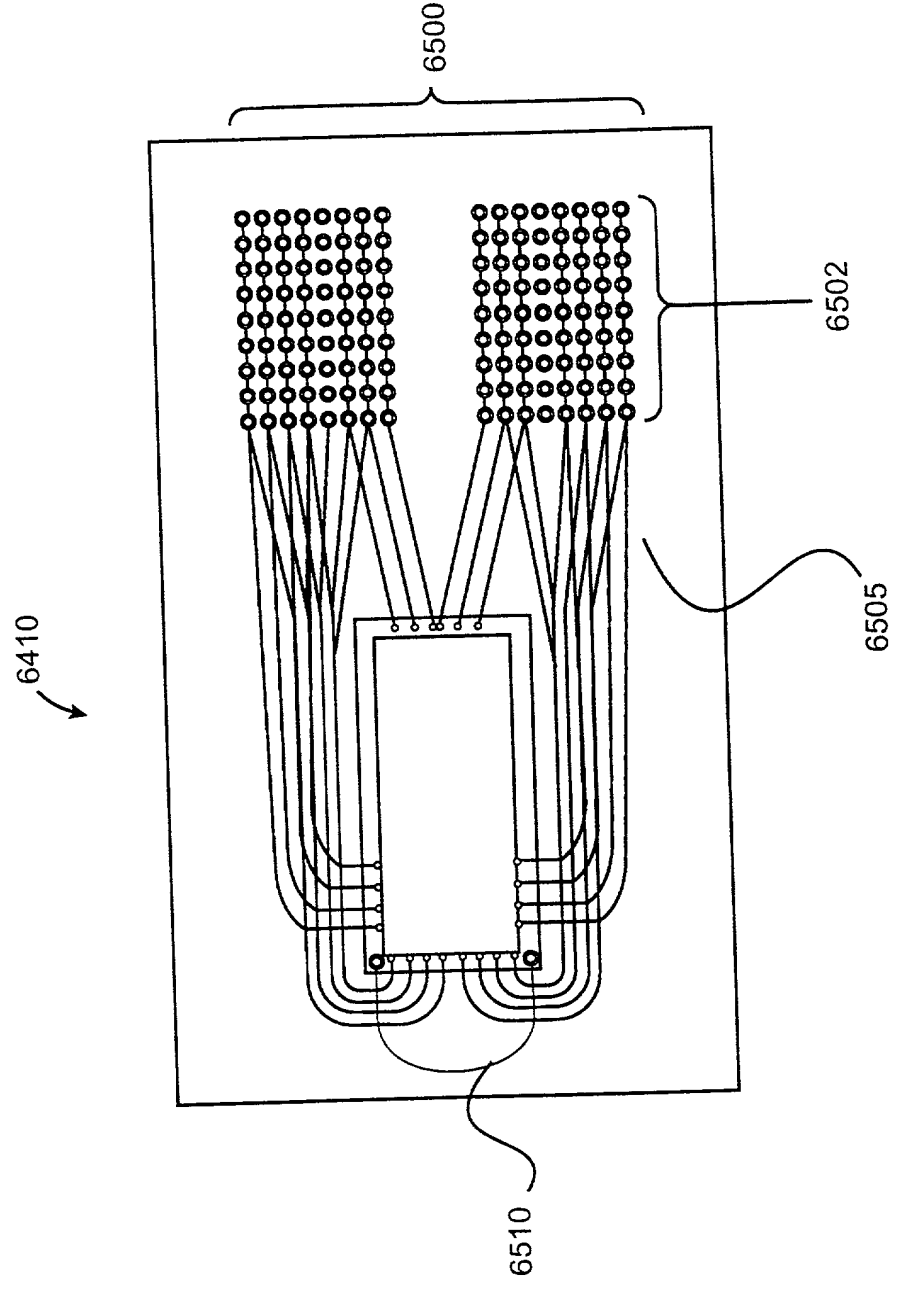
FIG. 54
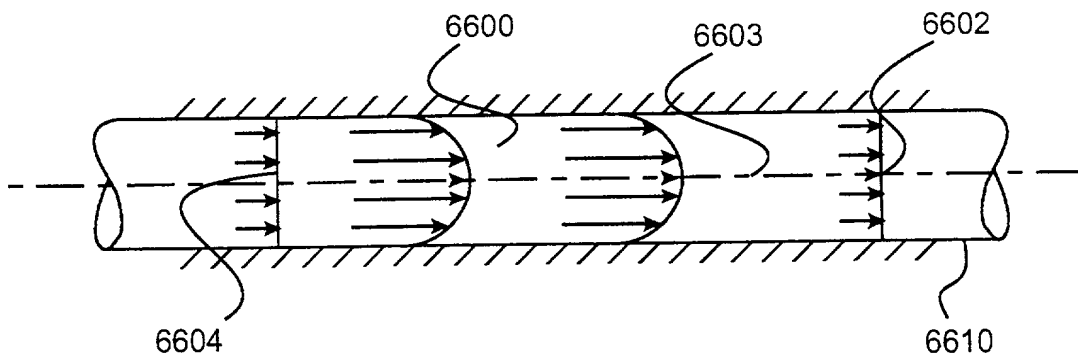
FIG. 55a
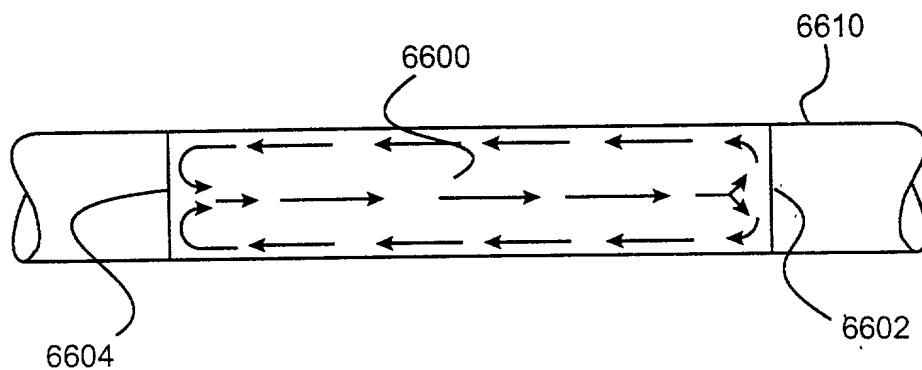
FIG. 55b

the genetic material of the cell. Further, the properties of these polypeptides, e.g., as enzymes, functional proteins, and structural proteins, are determined by the sequence of amino acids which make them up. As structure and function are

5 integrally related, many biological functions may be explained by elucidating the underlying structural features which provide those functions, and these structures are determined by the underlying genetic information in the form of polynucleotide sequences. In addition to encoding

10 polypeptides, polynucleotide sequences can also be specifically involved in, for example, the control and regulation of gene expression.

The study of this genetic information has proved to be of great value in providing a better understanding of life

15 processes, as well as diagnosing and treating a large number of disorders. In particular, disorders which are caused by mutations, deletions or repeats in specific portions of the genome, may be readily diagnosed and/or treated using genetic techniques. Similarly, disorders caused by external agents

20 may be diagnosed by detecting the presence of genetic material which is unique to the external agent, e.g., bacterial or viral DNA.

While current genetic methods are generally capable of identifying these genetic sequences, such methods generally

25 rely on a multiplicity of distinct processes to elucidate the nucleic acid sequences, with each process introducing a potential for error into the overall process. These processes also draw from a large number of distinct disciplines, including chemistry, molecular biology, medicine and others.

30 It would therefore be desirable to integrate the various process used in genetic diagnosis, in a single process, at a minimum cost, and with a maximum ease of operation.

Interest has been growing in the fabrication of microfluidic devices. Typically, advances in the

35 semiconductor manufacturing arts have been translated to the fabrication of micromechanical structures, e.g., micropumps,

microvalves, and the like, and microfluidic devices including miniature chambers and flow passages.

A number of researchers have attempted to employ these microfabrication techniques in the miniaturization of some of 5 the processes involved in genetic analysis in particular. For example, published PCT Application No. WO 94/05414, to Northrup and White, incorporated herein by reference in its entirety for all purposes, reports an integrated micro-PCR apparatus for collection and amplification of nucleic acids 10 from a specimen. However, there remains a need for an apparatus which combines the various processing and analytical operations involved in nucleic acid analysis. The present invention meets these and other needs.

15   SUMMARY OF THE INVENTION

The present invention generally provides miniature integrated fluidic systems for carrying out a variety of preparative and analytical operations, as well as methods of operating these systems and methods of using these systems.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic representation of a nucleic acid diagnostic system for analysis of nucleic acids from samples.

25   Figs. 2A and 2B show schematic representations of two alternate reaction chamber designs from a cut-away view.

Fig. 3 shows a schematic representation of a miniature integrated diagnostic device having a number of reaction chambers arranged in a serial geometry.

30   Figs. 4A-C show a representation of a microcapillary electrophoresis device. Figs. 4A and 4B show the microcapillary configured for carrying out alternate loading strategies for the microcapillary whereas Fig. 4C illustrates the microcapillary in running mode.

35   Fig. 5A illustrates a top view of a miniature integrated device which employs a centralized geometry. Fig. 5B shows a side view of the same device wherein the central

chamber is a pumping chamber, and employing diaphragm valve
structures for sealing reaction chambers.

Fig. 6 shows schematic illustrations of pneumatic
control manifolds for transporting fluid within a miniature
5   integrated device. Fig. 6A shows a manifold configuration
suitable for application of negative pressure, or vacuum,
whereas Fig. 6B shows a manifold configuration for application
of positive pressures. Fig. 6C illustrates a pressure profile
for moving fluids among several reaction chambers.

10   Fig. 7A shows a schematic illustration of a reaction
chamber incorporating a PZT element for use in mixing the
contents of the reaction chamber. Fig. 7B shows mixing within
a reaction chamber applying the PZT mixing element as shown in
Fig. 7A. Fig. 7C is a bar graph showing a comparison of
15   hybridization intensities using mechanical mixing, acoustic
mixing, stagnant hybridization and optimized acoustic mixing.

Fig. 8 is a schematic illustration of a side and top
view of a base-unit for use with a miniature integrated
device.

20   Fig. 10A is a gel showing a time course of an RNA
fragmentation reaction. Fig. 10B is a gel showing a
comparison of the product of an in vitro transcription
reaction in a microchamber vs. a control (test tube). Fig.
10C is a comparison of the PCR product produced in a PCR
25   thermal cycler and that produced by a microreactor.

Fig. 11 shows an embodiment of a reaction chamber
employing an electronic pH control system.

Figs. 12A-C show a schematic representation of a
miniature integrated device employing a pneumatic fluid
30   direction system utilizing a gas permeable fluid barrier bound
vents, e.g., a poorly wetting or hydrophobic membrane, and
pneumatically controlled valves. Fig. 12A shows an embodiment
of a single chamber employing this system. Fig. 12B is a
schematic illustration of a debubbling chamber for linking
35   discrete fluid plugs that are separated by a gas bubble. Fig.
12C schematically illustrates this system in an integrated
device having numerous chambers, including degassing chamber,

dosing or volumetric chamber, storage and reaction chambers. Fig. 12D is an illustration of an injection molded substrate which embodies the system schematically illustrated in Fig. 12C.

5   Fig. 13 is a schematic representation of a device configuration for carrying generic sample preparation reactions.

Fig. 14 is a schematic representation of a device configuration for carrying out multiple parallel reactions.

10   Fig. 15 shows a demonstration of integrated reactions in a microfabricated polycarbonate device. Fig. 15A shows the layout of the device including the thermal configuration of the device. Fig. 15B shows the results of PCR amplification and subsequent in vitro transcription within the chambers of

15   the device.

Fig. 16 schematically illustrates a deformable high capacity nucleic acid extraction device incorporating a porous material for extracting nucleic acids from samples.

Fig. 17 is a side sectional view of a miniaturized

20   reactor device incorporating a positive displacement fluid movement scheme.

Fig. 18A is a top plan view of the pneumatic portion of the reactor device of Fig. 17.

Fig. 18B is a top plan view of the fluid portion of the

25   reactor device of Fig. 17.

Fig. 19 schematically illustrates an affinity based nucleic acid extraction device incorporating a textured wall.

Fig. 20 illustrates an allele-specific purification device according to the present invention.

30   Fig. 21 is a schematic representation of a miniaturized device for performing rapid thermal cycling reactions, such as PCR or RT-PCR.

Figs. 22A and 22B are graphs of steady state power and cooling time versus thermal insulator thickness, respectively,

35   for the device of Fig. 21.

Fig. 23 is a top view of an array of thin-film heaters mounted on a single thermoelectric cooler for independent

rapid thermal cycling reactions in the miniature device of Fig. 21.

Fig. 24 is a cross-section view of a hybridization cartridge.

Fig. 25 is a schematic illustration of a sealed pneumatic cartridge having a deformable diaphragm for drawing fluid into or ejecting fluid from a chamber.

Fig. 26 schematically illustrates an array of sealed pneumatic chambers on disposable cartridges.

Fig. 27 is a cross-sectional view of an electrically controlled nucleic acid purification chamber.

Fig. 29 is a cross-sectional view of a miniaturized m-RNA purification system.

Fig. 30 is a sectional view of a cell lysis or nucleic acid fragmentization system incorporating acoustic energy.

Fig. 31 is a partial sectional view of a cartridge adapted for low volume hybridization of high density oligonucleotide arrays.

Figs. 34A-34E illustrate a system and method for linking two fluid plugs.

Figs. 35A and 35B illustrate alternative embodiments of the system of Figs. 34A-34E.

Figs. 38A, 38B and 38C illustrate a chamber adapted for measuring or metering a variable amount of fluid.

Figs. 39A-39E illustrate a method for measuring a fluid amount with the chamber of Figs. 38A and 38B.

Fig. 40 illustrates a tapered chamber for linking fluid plugs with surface tension.

Figs. 41A and 41B illustrate a stalactite chamber for linking fluid plugs with surface tension.

Figs. 42A and 42B illustrate a chamber having a shallow region for linking fluid plugs with surface tension.

Fig. 43A illustrates a previous fluid mixing/linking structure with a vent membrane.

Fig. 43B illustrate the inventive fluid mixing/linking structure with a tapered channel leading to the vent membrane.

Figs. 44A and 44B **REMOVED**